federal register

THURSDAY, MAY 13, 1976

PART V:

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

NEW ANIMAL DRUGS

Furazone (NF-180)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 510, 556 and 558]

[Docket No. 76N-0173; NADA No. 9-073 etc.]

FURAZOLIDONE (NF-180)

Withdrawal of Proposal and Advanced Notice of Proposed Rule Making

Elsewhere in this issue of the FEDERAL REGISTER, the Director of the Bureau of Veterinary Medicine of the Food and Drug Administration (FDA) is issuing a notice of opportunity for hearing on a proposal to withdraw approval of approved new animal drug applications (NADA's) Nos. 9-073, 9-393, 11-016, 11-405, 11-698, 11-810 (formerly FDC-D-281), and NADA Nos. 12-061, 13-805, and 32-319 for the use of furazolidone (NF-180) in food-producing animals on the grounds that the drug has been shown by appropriate tests to induce cancer when ingested by animals and there is no adequate, reliable and practicable method of analysis available for assuring that no residue of the drug will be found in any edible portion of such animals, as required by section 512(d)(1)(H) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(d)(1)(H)).

Consistent with this action, the Director is hereby withdrawing the proposal on furazolidone published in the FEDERAL REGISTER of August 4, 1971 (36 FR 14335), and is proposing to revoke §§ 510.515 (a)(4) and (5), (b)(15) and (17)(ii), (c)(8), (9) and (10); 556.290; 558.15(g)(1) and (2); and 558.262. Comments by June 14, 1976.

The Director has reviewed the potential environmental impact of the proposal and has concluded that the proposed action would not significantly affect the quality of the human environment. An environmental impact analysis report and environmental impact assessment are on file in the office of the Hearing Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20852.

The Director has carefully considered the inflation impact of this proposed action. A major inflation impact has been found, as defined in Executive Order 11821, OMB Circular A-107, and the Guidelines issued by the Department of Health, Education, and Welfare. Copies of the inflation impact statement are on file with the Hearing Clerk, Food and Drug Administration. The major conclusions found in the inflation impact statement are:

(1) There are no satisfactory alternatives to the agency's proposed action which are consistent with the legal constraints imposed by the Federal Food, Drug, and Cosmetic Act, as amended.

(2) Poultry production, i.e., broilers, turkeys, and eggs (as affected through diseased or poorer quality replacement birds), is estimated to decrease anywhere from 2 to 20 percent, and swine production is estimated to decrease from 0 to 5 percent following a furazolidone ban. The lower estimate are more likely for the first year following the ban, but in subsequent years the higher estimates are expected to apply because the resistance of poultry and swine to antibiotics and sulfa drugs will probably become a more acute problem with respect to the prevention and treatment of poultry and swine diseases.

(3) The ranges of decrease in poultry and swine production and their resultant impact on retail prices will lead to an annual increase in cost to consumers of between just less than $1.50 per capita to just more than $12 per capita for consumption of poultry and swine products if producers do not adjust by feeding more poultry and swine in order to offset losses from morbidity, mortality, and condemnation. The per-capita cost annual increases are equivalent to an aggregate ranging from $291 million to $2.635 billion.

(4) Again depending on the resistance of poultry and swine to antibiotics and sulfa drugs, if poultry and swine producers expanded operations to overcome 100 percent of the production losses from morbidity, mortality, and condemnation, then the feed plus nonfeed costs for this expansion would range from $164 million to $1.8 billion annually. Assuming these entire costs are passed on to consumers, this amounts to between about $.80 per capita and almost $9 per capita annually.

(5) A ban on furazolidone may cause a decrease in the supply of poultry amounting to 20 percent and swine amounting to 5 percent within the next 2 years if resistance to drugs other than furazolidone is substantial for poultry and swine, and producers do not feed more swine and poultry in order to offset losses from morbidity, mortality, and condemnation.

(6) A ban on furazolidone may cause major inflation impacts, as defined by the OMB/HEW criteria, in the area of competition for the poultry and swine industries.

(7) A ban on furazolidone would not cause major inflation impacts as defined by the OMB/HEW criteria, in the area of employment.

(8) A ban on furazolidone would not cause major inflation impacts, as defined by the OMB/HEW criteria, in the areas of national productivity or use of energy.

(9) The benefits from implementing the proposed action will be the elimination of any risk of any cancer associated with the consumption, via the edible tissues or products from food-producing animals, of residues attributable to furazolidone.

Therefore, pursuant to the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343-361 (21 U.S.C. 360b)) and under authority delegated to the Commissioner (21 CFR 2.120), and subdelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 2.120(m)), the Director proposes to revoke the following regulations in whole or in part insofar as they apply to furazolidone (NF-180): §§ 510.515 (a)(4) and (5), (b)(15) and (17)(ii), (c)(8), (9) and (10); 556.290; 558.15(g)(1) and (2); and 558.262.

Interested persons may, on or before June 14, 1976, submit to the Hearing Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20852, written comments (preferably in quintuplicate and identified with the Hearing Clerk docket number found in brackets in the heading of this document) regarding this proposal. Received comments may be seen in the above office during working hours, Monday through Friday.

Dated: May 10, 1976.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.76-13968 Filed 5-10-76;2:04 pm]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

[Docket No. 76N-0172; NADA No. 9-073 etc.]

HESS AND CLARK ET AL.

Furazolidone (NF-180); Notice of Opportunity for Hearing on Proposal To Withdraw Approval of Certain New Animal Drug Applications

The Director of the Bureau of Veterinary Medicine of the Food and Drug Administration (FDA) is issuing a notice of opportunity for hearing on a proposal to withdraw approval of approved new animal drug applications (NADA's) Nos. 9-073, 9-393, 11-016, 11-405, 11-698, 11-810, (formerly FDC-D-281) and NADA Nos. 12-061, 13-805, and 32-319 for the use of furazolidone (NF-180) in food-producing animals on the grounds that the drug has been shown by appropriate tests to induce cancer when ingested by animals and there is no adequate, reliable and practicable method of analysis available for assuring that no residue of the drug will be found in any edible portion of such animals, as required by section 512(d)(1)(H) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b (d)(1)(H). Holders of approved NADA's have until June 14, 1976 to submit requests for hearing in accordance with § 514.200 (21 CFR 514.200). Elsewhere in this issue of the FEDERAL REGISTER, the Director is issuing an advanced notice of proposed rule making to revoke the appropriate provisions of sections affected by the proposed withdrawal.

Furazolidone (NF-180) is a member of a class of chemicals called "nitrofurans" which are used at low levels in animal feed as antibacterial and antiprotozoan agents to increase resistance to disease, thereby assisting growth. Nitrofurans also have prescribed therapeutic uses. In addition to furazolidone (NF-180) which is the subject of this notice, there are three other nitrofurans used in food-producing animals which will be subject to subsequent agency action: nitrofurazone (NF-7), nihydrazone (NF-64), and furaltadone (NF-260). A fifth nitrofuran, furamazone (NF-84), is not at this time the subject of contemplated agency action.

Nitrofurazone (NF-7), approved in 1948, was the first nitrofuran approved by FDA for use in food-producing animals. It is currently approved for use in mastitis products for dairy cattle (dry cow treatment only), suppositories for vaginal infections in large animals, and feed medication in swine for the treatment of bacterial enteritis, in chickens and turkeys for prevention of coccidiosis, and in chickens for prevention of pullorum.

The initial NADA for furazolidone (NF-180) was approved in 1953. The drug is currently approved for use in poultry for the prevention and treatment of fowl typhoid, paratyphoid, and pullorum in chickens and turkeys; blackhead (histomoniasis) in chickens and turkeys; infectious hepatitis in chickens; hexamitiasis in turkeys; the prevention and control of air-sac infection (associated with *Escherichia coli*), nonspecific enteritis (blue comb, mud fever), ulcerative enteritis (quail disease), synovitis (arthritis due to filterable virus) in chickens and turkeys; the prevention and treatment of paracolon infection (*Paracolobactrum*) in chickens and turkeys; coccidiosis (*E. acervulina, E. necatrix, E. tenella*) in chickens; and for growth and feed efficiency in chickens and turkeys. It is used in swine for the prevention and treatment of bacterial enteritis (necrotic enteritis, necro, black scours), or vibrionic (bloody) dysentery; and for growth promotion and feed efficiency.

Furaltadone (NF-260) was approved in 1962 for treatment of mastitis in dairy cattle through injections into the mammary gland.

Nihydrazone (NF-64) was approved in 1963 for use in medicated chicken feeds for prevention of a number of diseases similar to those listed above for furazolidone (NF-180).

In February and May 1964, two scientists at the University of Wisconsin began studies on the toxicity to rats of several nitrofurans including nitrofurazone (NF-7). By the end of February 1965, both studies showed that a substantial number of mammary tumors had developed in the rats fed nitrofurazone (NF-7). Of 44 rats exposed to nitrofurazone (NF-7), 35 developed multiple mammary tumors. Although the results did not indicate induction of cancer, they did raise the question of the carcinogenic potential of nitrofurans. In April 1965, the University of Wisconsin scientists met with representatives of Norwich Pharmacal Co., Division of Morton-Norwich Products, Inc., the sole American manufacturer of the four nitrofurans used in food-producing animals, to discuss their findings regarding nitrofurazone (NF-7). At the suggestion of Norwich, the scientists notified FDA of the preliminary results of the study.

In June 1965, FDA, the National Cancer Institute of the Department of Health, Education, and Welfare, Norwich, and the University of Wisconsin scientists met to discuss the findings of the University scientists. Following the discussions, Norwich began two studies of the effect of long-term feeding of nitrofurans including furazolidone (NF-180), furaltadone (NF-260), nitrofurazone (NF-7), and nihydrazone (NF-64) to rats. Reports on these two studies were prepared in October 1966, and January 1967, and submitted to the Bureau of Veterinary Medicine as part of a June 2, 1967 "Drug Experience Report." Copies of these studies have been placed on file with the Hearing Clerk, Food and Drug Administration.

In the first of the Norwich studies, 245 female Sprague-Dawley (Holtzman strain) rats were divided into 7 test groups of 35 rats each. A control group was fed a drug-free diet for 53 weeks. Each of the other groups was fed a diet containing one of the nitrofurans for 45 weeks and a drug-free diet for 8 additional weeks before the animals were sacrificed. The study showed that rats fed the nitrofurans had a significantly higher incidence of mammary tumors compared to control rats. The relative mammary-tumor-inducing potency of the nitrofurans in this study in decreasing order was furaltadone (NF-260) >furazolidone (NF-180) >nitrofurazone (NF-7) >nihydrazone (NF-64).

The second Norwich study used 280 Carworth Farms (CFE strain) rats which were divided into 7 test groups with 20 males and 20 females in each test group. A control group was fed a drug-free diet for 52 weeks. Each of the other groups was fed a diet containing one of the nitrofurans for 45 weeks and a drug-free diet for 7 additional weeks. This study also showed a higher incidence of mammary tumors in the female rats in each of the groups fed the nitrofurans when compared to female rats in the control group and indicated the same relative tumor-inducing potency seen in the female Sprague-Dawley rats. No significant effects on tumor development were noted in the male rats.

In February 1967, Hess & Clark, currently a Division of Rhodia, Inc., which markets the four nitrofurans used in food-producing animals, submitted a "Drug Experience Report" to the Bureau of Veterinary Medicine containing a report of a 2-year chronic toxicity study of furazolidone (NF-180) in rats. In this study, 60 rats were divided into 3 groups, each consisting of 10 male and 10 female rats. One group was fed a furazolidone-free control diet; the diets for the other groups contained two levels of furazolidone (NF-180). Results of the study showed that there were three times as many tumors in rats fed furazolidone (NF-180) at a level of .01% in the diet when compared to rats in the control group.

In June 1969, FDA submitted the question of the carcinogenicity of nitrofurans to the Interdepartmental Technical Panel on Carcinogens (the Panel) for review. The Panel incuded representatives from FDA, the National Cancer Institute, and the U.S. Department of Agriculture. After reviewing the studies submitted by the University of Wisconsin scientists, Norwich, and Hess & Clark, the Panel reported to the Commissioner of Food and Drugs on August 14, 1969, that it had concluded that the four nitrofurans used in food-producing animals induce mammary tumors, but that evidence was inadequate to make a definitive decision on the potential of these nitrofurans to induce cancer. The Panel recommended that chronic toxicity studies of at least 18 months duration in male and female rats and mice be performed, that studies be performed on the storage and localization of the drug and its metabolites as a function of time in rats and mice, and that regulations be issued requiring that the nitrofurans be withdrawn from animal feed for 21 days before slaughter to prevent transmission of metabolites to humans.

On August 26, 1969, the Bureau of Veterinary Medicine asked Norwich by letter for additional data on metabolites of furazolidone (NF-180) in food-producing animals. Norwich informed the Bureau by letter in October 1969, that it was working on a method of analysis

for two postulated furazolidone metabolites, NF–362 and NF–682, and promised to submit the data as soon as the methods were completed.

Because the Panel had been unable to determine whether nitrofurans are carcinogenic, an Ad Hoc Committee (the committee) was established by the FDA in December 1969. The committee was composed of one member of the Eppley Institute for Research in Cancer, University of Nebraska, and two members from the National Cancer Institute, one of whom had been a member of the Panel. Since the question posed to the committee was whether nitrofurans are carinogenic, the committee did not review the residue data in food-producing animals available at that time. No formal committee report was issued evaluating the existing tumorigenic data on nitrofurans, but each member submitted a separate report. The two committee members from the National Cancer Institute advised FDA by letter in January 1970 that nitrofurans should not be ingested by people for any extended period of time until additional chronic toxicity testing had been completed. They approved of the continued use of the drugs in animals with the 5-day withdrawal period providing data were available to show that residues were negligible. On June 5, 1970, the committee member from the Eppley Institute notified FDA that he had concluded that all four nitrofurans were carcinogenic.

During 1970, FDA reviewed the toxicity and residue studies submitted by the University of Wisconsin scientists, Norwich, and Hess & Clark, together with the recommendations of the Panel and the committee.

In January 1971, Norwich and Hess & Clark submitted supplemental NADA's for their nitrofuran products requesting that the labels be amended to provide withdrawal periods for swine, turkeys, and chickens, and longer withdrawal periods for dairy cattle. The companies proposed that the regulations be amended to require that (1) swine, turkeys, and chickens be withdrawn from furazolidone (NF–180) and nitrofurazone (NF–7) 5 days before slaughter, (2) chickens be withdrawn from nihydrazone (NF–64) 4 days before slaughter, and (3) dairy cattle be withdrawn from furaltadone (NF–260) 48 hours before their milk is used for food purposes. The supplemental NADA's also proposed that several uses of nitrofurans be eliminated. Use of furazolidone (NF–180) in the feed of laying hens and rabbits was deleted from furazolidone (NF–180) labels, and a warning not to feed furazolidone (NF–180) to replacement chickens, i.e., those chickens being raised to become laying hens, over 14 weeks of age was added to the labels.

The Bureau of Veterinary Medicine told Norwich and Hess & Clark that under current agency policy it could not approve the supplemental NADA's until the question of the safety of nitrofurans was resolved, but it agreed to the use of the revised labels in the interim. Hess & Clark and Norwich informed FDA by letters dated January 7 and 8, 1971, respectively, that they were revising the labels of their nitrofuran products in accordance with the proposed supplemental NADA's. Norwich also made a commitment to develop a method for detecting nitrofuran residues that would be sensitive to 2 parts per billion (ppb) in edible tissue and 0.2 ppb in milk.

The Food and Drug Administration issued notices of opportunity for hearing, published in the FEDERAL REGISTER of March 31, 1971 (36 FR 5926 and 5927) proposing to withdraw approval of NADA's for nihydrazone (NF–64) and nitrofurazone (NF–7). Each of the notices stated:

> Information available to the Commissioner establishes that the drugs, when administered to laboratory animals, have been shown to produce tumors. The drugs are, therefore, not considered to be safe for use in the absence of appropriately sensitive methods of analysis to establish their absence in food derived from treated animals.

Joint requests for hearing on both nitrofurazone (NF–7) and nihydrazone (NF–64) were filed by Norwich and Hess & Clark. In addition, Pharm-House, Inc., submitted a method for the determination of trace amounts of nitrofurazone (NF–7) in milk which it indicated could detect nitrofurazone (NF–7) levels down to 2 ppb. Because milk may be the total daily diet of infants, FDA does not consider 2 ppb adequately sensitive for the detection of nitrofurazone (NF–7) in milk. Therefore, this method has not been evaluated.

The Food and Drug Administration issued notices of opportunity for hearing, published in the FEDERAL REGISTER of August 4, 1971 (36 FR 14343) proposing to withdraw approval of NADA's for furazolidone (NF–180) and furaltadone (NF–260). The reason for the proposals was the same as that stated in the March 31, 1971 notices for nitrofurazone (NF–7) and nihydrazone (NF–64), quoted above.

In response to these notices, the Director of Health Services for Perdue, Inc. submitted a letter in which he stated, "Furazolidone is one of the most, if not the most, important single drug available to the poultry industry; for which there is no suitable substitute for certain diseases." The Director's concern is discussed in the FDA inflation impact statement summarized in the proposal published elsewhere in this issue of the FEDERAL REGISTER.

Norwich and Hess & Clark responded to the August 4, 1971, notices by again filing requests for hearing. Norwich assured that it had adequate methods of analysis to determine residues and adequate data to show that no residue would result under the currently labeled conditions of use.

In addition, Norwich set forth a hypothesis that nitrofurans were not primary tumorigens but stimulated spontaneous tumor growth by disrupting the normal hormone balance.

Norwich has continued to submit data pertaining to the use of furazolidone (NF–180) in food-producing animals. The Food and Drug Administration has determined that the new data demonstrate that furazolidone (NF–180) is carcinogenic in test animals and that the firm has failed to submit an adequate method of analysis to assure the absence of residues in food for humans. The Director is withdrawing the August 4, 1971 notice of opportunity for hearing on the proposed withdrawal of approval of certain uses of furazolidone (NF–180) on grounds that it was tumorigenic and therefore unsafe, and is issuing this new notice proposing withdrawal of approval under section 512(d)(1)(H) of the Federal Food, Drug, and Cosmetic Act, on grounds that it is carcinogenic and no adequate, reliable, and practicable method of analysis is available for monitoring food to assure that there are no residues attributable to the use of furazolidone (NF–180).

Section 512(d)(1)(H) of the act requires that FDA shall refuse to approve a drug if the Secretary finds that "such drug induces cancer when ingested by man or animal or, after tests which are appropriate for the evaluation of the safety of such drug, induces cancer in man or animal * * *." However, section 512(d)(1)(H) of the act permits approval of the use of a carcinogen as a drug in animals if the Secretary (by delegation under § 2.120 (21 CFR 2.120) to the Commissioner of Food and Drugs) finds that:

> * * * under the conditions of use specified in proposed labeling and reasonably certain to be followed in practice (i) such drug will not adversely affect the animals for which it is intended, and (ii) no residue of such drug will be found (by methods of examination prescribed or approved by the Secretary by regulations * * *), in any edible portion of such animals after slaughter or in any food yielded by or derived from the living animals * * *.

On the basis of all the data now available to him, including the data available to FDA when the applications were approved, and the data subsequently received on the basis of which the 1971 notice was issued, and the new data submitted since 1971 by Norwich, the Director has concluded that the continued use of furazolidone (NF–180) in food-producing animals is contrary to section 512(d)(1)(H) of the act in that:

(1) Furazolidone (NF–180) is carcinogenic. The drug has been shown to induce cancer when ingested by animals.

(2) Adequate, reliable, and practicable methods of analysis are not available for monitoring food nor can conditions of use be specified in the labeling to assure that there are no residues attributable to the use of furazolidone (NF–180).

The data on which these conclusions are based are presented in the succeeding sections of this notice.

I. FURAZOLIDONE IS CARCINOGENIC

Data submitted by Norwich since 1971 include the following four chronic toxicity studies designed to assess the tumorigenic effects of furazolidone (NF–180) in laboratory animals. These studies include two high-dose lifetime feeding

studies in the Sprague-Dawley and Fischer rats using comparable protocols, a lower dose 2-year feeding study in the Sprague-Dawley rat, and a study using the Swiss MBR–ICR mouse.

(1) The High-Dose Sprague-Dawley Rat Study. "Tumorigenic Evaluation of NF–180 in Sprague-Dawley and Fischer Rats, Part I, Sprague-Dawley Evaluation," November 9, 1973, Project No. 475.09D, NADA 9–393, Vol. 10. From cover letter to FDA dated November 12, 1973.

(2) The Fischer Rat Study. "Tumorigenesis Evaluation of NF–180 in Sprague-Dawley and Fischer Rats, Part II, Fischer 344 Evaluation," January 31, 1974, Project No. 475.09D, NADA 9–393, Vol. 16. From cover letter to FDA dated February 8, 1974.

(3) The Low-Level Sprague-Dawley Rat Study. "Chronic Toxicopathologic Safety Study (Two-years of NF–180 in Rats)," Final Report, November 9, 1973, Project No. 475.09C, NADA 9–393, Vol. 12. From cover letter to FDA dated November 12, 1973.

(4) The Mouse Study. "Tumorigenesis Evaluation of NF–180 in Mice," (23 months) Final Report, Norwich Pharmacal Co., January 31, 1974, Project No. 375.09E, NADA 9–393, Vol. 18. From cover letter to FDA dated February 8, 1974.

Tumors develop spontaneously in animals, including humans, as they age. To determine if the development of tumors is related to a drug exposure, rats are divided into different groups, all but one of which receive different dose levels of the drug. Statistical analyses are performed on the data to see whether there is an increased incidence in the number of animals that develop tumors in any one of the treated groups compared to untreated animals (controls), and to see if there is an increase in the number of tumors in each of the treated animals (tumor multiplicity) as compared to controls.

As further evidence that any statistically significant difference in tumors in any treatment group as compared to controls is drug-related, the dose-response relationship is examined and mathematically described. In this examination the results from all dose groups are statistically used to see if increased amounts of drug exposure result in a corresponding increase in tumor development. If it does, this is considered a significant dose-response. When the dose-response relationship is significant and, in addition, there are no significant departures from linearity, this is conclusive evidence that there is drug-related tumorigenicity.

The statistical analyses on tumors are performed to determine if there are differences related to the following: sex, malignancies, incidence based on all tumors (malignant, benign, or malignant and benign combined), and tumor multiplicity either in malignancies alone or all tumors combined.

Since drugs may induce the concurrent development of both benign and malignant tumors, analysis of change in benign tumors alone with dose may provide additional supporting evidence that a neoplastic response at low doses is related to carcinogenic properties of the compound that are only evident in the bioassay at higher doses.

A tumorigen may result in the development of a sepcific type of tumor or tumors at a specific body site, or the tumorigenic response may be one of a general increase in tumors, not type- or site-specific (random tumor induction). Therefore, the statistical analysis may also include a comparison of subgroups of data to determine if, for example, there is a site-specific effect without an effect on random tumor induction.

Mortality is also examined to determine if the drug has an effect on lifespan shortening. Since the development of cancer involves a period of latency, and dose is important not only in whether or not cancer occurs but also in the length of the latency period, an effect on mortality must be taken into consideration in the overall interpretation of results. Of course, shortening of lifespan alone, whether or not it is related to the development of tumors, is a significant effect not to be overlooked in the toxicological evaluation.

A discussion of the four chronic toxicity studies follows. In each study, all tumor results were based on histopathology findings unless otherwise specified. In the discussion, the terms "induce" and "significant" are used only if the conclusion is based on statistical evidence at a confidence level of 95% or better. Although the term "tumor" has broad meaning including any swollen mass, in this notice the term "tumor" is used only as a synonym for "neplasm" and designates any new or abnormal growth. When the term "tumor" is used without a modifier to designate whether the neoplasm is benign or malignant, the term means all tumors combined, i.e., malignant, benign, or both benign and malignant in the same animal. In most cases, animals with malignant tumors also have benign tumors.

(1) *The High-Dose Sprague-Dawley Rat Study.* Four hundred Sprague-Dawley rats of the Charles River strain approximately 2 months of age were divided into 4 groups of 50 male and 50 female rats each. A control group was fed a furazolidone-free diet. The diet of the three other groups contained furazolidone in the feed at levels of 0.025% (250 parts per million (ppm)), 0.05% (500 ppm), and 0.1% (1,000 ppm) for approximately 18 months (553 days). All groups of rats were then maintained on the furazolidone-free diet until mortality in each group reached 90%, at which time the remaining 10% were sacrificed. This was approximately 13 months after the drug was withdrawn from the diets of the animals.

In this study, and also in the other two rat studies, there was a marked sex difference in the development of tumors and types of tumors. The primary tumor effect observed in the Sprague-Dawley female rat was in the mammary glands. Furazolidone induced malignant mammary tumors in the female rats. There was also a concurrent induction of benign mammary tumors. There were increases in both the numbers of female rats with mammary tumors and the number of mammary tumors per animal (tumor multiplicity). In the male rats no significant effects were observed in the mammary gland, but there was an induction of random nonmammary tumors with no one type singularly significant. No significant drug-related effects based on the analysis of malignancies alone were seen in the male rats. Furazolidone had a significant effect on mortality, reducing the lifespan of both the male and female rats.

The relevant facts related to these findings are as follows:

The number of female rats with malignant mammary tumors increased as the dose levels increased, and the dose-response relationship was linear and significant ($p<.01$). At the highest dose level (0.1%), the proportion of female rats with malignant tumors was significantly increased ($p<.05$) when compared with the control rats on the furazolidone-free diet.

Furazolidone also concurrently induced benign mammary tumors in female rats. When the proportion of treated rats with only benign tumors was compared with controls, the increase was significant at the 0.025% dose level ($p<0.2$) and the 0.05% dose level ($p<.01$). However, no significant increase occurred in the high-dose group where the increase in the proportion of rats with malignancies was signficant. The proportion of female rats with only benign mammary tumors showed a significant regression with log dose, but there were departures from linearity due to the tumor response at the high dose.

At all dose levels, the proportion of female rats with mammary tumors was significantly increased ($p<.05$) when compared with controls. The analysis on multiplicity of mammary tumors in female rats indicated a significant increase in the proportion of rats with multiple mammary tumors for the low and mid-dose levels ($p<.05$) compared to controls, when pair-wise chi-square tests were conducted for animals developing two or more, three or more, and four or more tumors.

Although furazolidone did not induce tumors in the mammary glands of male rats, it did cause a random induction of tumors in other parts of the body. The proportion of male rats which developed nonmammary tumors at the 0.1% dose level was significantly increased ($p<.05$) compared to controls. The nonmammary tumor development showed a significant linear dose response ($p<.05$). The nonmammary tumors included squamous cell carcinoma, dermal fibroma, pituitary neoplasms and lymphoreticular neoplasms, including such cancers as leukemia and lymphosarcomas, with no one type or site singularly significant.

Furazolidone had a drug-related effect on mortality. An assessment of mortality when furazolidon treatment was discontinued after 18 months revealed a significant increase ($p<.05$) at the 0.05%

and 0.1% dose in males and at the 0.1% dose in females. At 26 months there was a significant effect on early mortality in males at the 0.1% dose ($p<.05$) and in females at the 0.05% dose ($p<.01$) and at the 0.1% dose ($p<.02$).

The Director concludes on the basis of these results that furazolidone is a demonstrated carcinogen in the Sprague-Dawley rat, based on the significant increase in the proportion of female rats at the 0.1% dose level with malignant mammary tumors as compared to controls, and the significant linear dose response in malignant mammary tumor development. He further concludes that the tumorigenic effects of furazolidone are not limited to the mammary gland but include effects on the combined increase in benign and malignant nonmammary tumors in male rats. He also concludes that furazolidone resulted in early mortality in both male and female rats.

(2) *The Fischer Rat Study.* Four hundred rats of the Fischer 344 strain, approximately 2 months of age, were divided into 4 groups of 50 male and 50 female rats each. A control group was fed a furazolidone-free diet. The diet of the three other groups contained furazolidone in the feed at levels of 0.025% (250 ppm), 0.05% (500 ppm), and 0.1% (1,000 ppm) for 20 months. All groups of rats were then maintained on the furazolidone-free diet until mortality in each group reached 90%, at which time the remaining 10% were sacrificed. This was approximately 11 months after the drug was withdrawn from the diets of all the animals.

In this study, as in the other two rat studies, there were sex differences in the development of tumors and types of tumors. The primary effect seen in the Fischer female rats was the development of tumors in the mammary gland. Furazolidone induced malignant mammary tumors in the female rats at the highest dose and had a significant effect on both the number of female rats which developed mammary tumors and in the multiplicity of mammary tumors in the female rats as compared to controls. Furazolidone also induced other specific nonmammary tumors in both males and females including tumors in the thyroid and sweat glands. Although no mammary tumor effect occurred in the male rats, there was an effect at the highest dose on the occurrences of mesothelioma in the scrotal sack and in the appearance of basal cell epitheliomas. Mesothelioma are cancerous cells of the body cavity linings, e.g., pleura, peritoneum, and pericardium. In the male these cancerous cells tend to gravitate to the scrotal cavity where their detection is generally most readily observed. Basal cell epitheliomas are skin tumors and low grade cancers which usually do not metastasize. There was also a drug-related effect on mortality in both sexes.

The relevant facts related to these findings are as follows:

The proportion of female rats with malignant mammary tumors at the 0.1% dose level was significantly increased ($p<.05$) over controls. Malignant mammary tumors were diagnosed only in female rats given the highest dose of furazolidone.

At all dose levels, the proportion of female rats with mammary tumors was significantly increased ($p<.01$) over controls. There was a significant relationship between the amount of dose and the proportion of rats with mammary tumors. However, there was significant departure from linearity in that there was a leveling off of the effect at the high-dose level, possibly due to increased mortality. The proportion of female rats with multiple mammary tumors was significant ($p<.01$) for all treatment groups compared to controls.

There was no significant difference in the proportion of furazolidone-treated male rats with mammary tumors when compared with controls.

There were other specific tumors in males and females that were significantly increased ($p<0.05$) by furazolidone exposure. Thyroid adenomas were significantly increased in both males and females at the 0.05% and 0.1% dose levels. Sebaceous adenomas were significantly increased in males at the 0.05% dose level and in both males and females at the 0.1% dose level. Testicular mesotheliomas and basal cell epitheliomas were significantly increased in males at the 0.1% dose level.

Furazolidone had an effect on mortality which was significant ($p<.05$) in the females at the 0.1% dose level and in the males at the 0.05% and 0.1% dose levels. The dose-response relationship on early mortality was significant and linear for both males and females.

The Director concludes on the basis of these results that furazolidone is a demonstrated carcinogen in the Fischer rat based on the significant increase in the proportion of female rats at the 0.1% dose level with malignant mammary tumors and the significant increase in testicular mesotheliomas in the male rates at the 0.1% dose level as compared to controls. He also concludes that furazolidone resulted in early mortality in both male and female rats.

(3) *The Low-Level Sprague-Dawley Rat Study.* Three hundred twenty Sprague-Dawley rats of the Charles River strain approximately 2 months old were divided into 4 groups of 40 male and 40 female rats each. A control group was fed a furazolidone-free diet. The treated rats were given furazolidone in the feed at dose levels of 1, 5, 15 milligrams/kilograms of body weight/day. The concentration of furazolidone in the diet was increased as the animals continued to grow. From the 266th day on, the dose levels in the diet were 0.0025% (25 ppm), 0.0125% (125 ppm), and 0.0375% (375 ppm). Drug treatment was continuous throughout the experiment, which was terminated after 2 years (731 days).

Furazolidone treatment affected the total development of tumors in the female Sprague-Dawley rats, which included increases in both the numbers of female rats with tumors and in the multiplicity of tumors in the rats as compared with controls. Although at the end of the experiment there were no significant effects in either males or females in the number of rats with tumors based on any one tumor site to type alone, the data on tumor palpation (examination by touch and feel) indicated that furazolidone increased the onset time of mammary tumor development in the female rat. No drug effect on tumor development at the dose levels in this study occurred in the male rats. In addition to the induction of tumors, furazolidone had a significant effect on mortality in the female rats.

The relevant facts related to these findings are as follows:

There was a significant ($p<.05$) linear dose-response relationship for the number of female rats with tumors, with a significant increase ($p<.05$) in the proportion of female rats at the 0.0375% dose level with tumors in comparison with females in the control group. The majority of tumors observed were in the mammary gland. In addition to the mamary tumors, pituitary adenomas and lymphoreticular neoplasms, including leukemia, lymphosarcoma and reticulum cell sarcoma, were diagnosed.

An analysis of the incidence of multiple mammary tumors in female rats showed a significant increase ($p<.05$) for both the 0.0125% and 0.0375% dose groups compared to controls with a significant linear dose-response relationship ($p<.05$).

Based on the sponsor's data of tumor palpation prior to termination of the experiment, furazolidone caused early onset time of mammary tumor development in the female rats. At 16 months there was a significant increase ($p < .05$) in the number of female rates with mammary tumors at the 0.0375% dose level compared with controls. There was also a significant linear dose response for mamary tumor development in females over the first 16 months of the study.

There were no significant differences based on the analysis of malignant tumors alone in either male or female rats in this study.

Mortality for the first 20 months was significantly ($p<.05$) increased in females at the 0.0375% dose level when compared to the controls. For the 24-month period, mortality was significantly ($p<.05$) increased in female rats at the 0.0125% and 0.0375% dose levels compared to controls. The dose-response effect on mortality in the female rats was significant ($p<.05$) with no significant departures from linearity. No significant differences in mortality were seen in the male rats.

Norwich claims this study demonstrate that the 0.0025% level of exposure was a "no-effect" dose for the female rats and that 0.0375% was a "no-effect" dose for the male rats.

The Director concludes from the results of this study that furazolidone has a significant linear dose-related effect in female rats with respect to the proportion of rats with tumors of all types combined, the incidence of multiple mammary tumors, and early mortality. For this reason, the Director rejects Norwich's claim that the 0.0025% level of exposure

was a "no-effect" dose for the female rats. He concludes that a more conservative approach to the estimation of a "no-effect" level of exposure is required.

(4) *The Mouse Study.* Four hundred Swiss MBR/ICR mice approximately 2 months of age were divided into 4 groups of 50 male and 50 female mice each. A control group was fed a furazolidone-free diet. The diet of the three other groups contained furazolidone in the diet at levels of 0.0075% (75 ppm), 0.015% (150 ppm), and 0.03% (300 ppm) respectively for 18 months. All groups of mice were then maintained on a furazolidone-free diet for an additional 10 months before the study was terminated.

In contrast to the rat studies, no sex differences were seen in the effect of furazolidone on tumor development in male and female mice. Furazolidone induced malignant tumors in both male and female mice. Malignant lung tumors (bronchial adenocarcinomas) were the primary effect. Furazolidone also resulted in early mortality in both sexes.

The findings on which these conclusions are based are as follows:

The proportion of male mice at the 0.015% and 0.03% dose and the proportion of female mice at the 0.03% dose with bronchial adenocarcinomas was significantly increased compared to controls. In both sexes the incidence of mice with bronchial adenocarcinomas showed a significant linear dose response ($p<.01$) with no departures from linearity.

The dose-response relationships for the incidence of mice with malignancies of all types and also for the incidence based on all tumors were significant ($p<.05$) for both sexes with no significant departures from linearity. There was a significant increase ($p<.05$) in both the development of malignancies and in the development of benign and malignant tumors combined in the male mice at the 0.03% and 0.015% dose levels and in the female mice at the 0.03% dose when compared to controls.

The proportion of male and female mice with more than one tumor each was analyzed. The males at the 0.03% dose level had significantly more ($p<.05$) multiple tumors than the controls and there was a significant linear dose-response relationship for tumor multiplicities found in the males. There were no significant differences in tumor multiplicity for the females.

Furazolidone had an effect on early mortality in the mice. The males at the 0.03% dose level and the females at the 0.015% and 0.03% dose levels differed significantly ($p<.05$) in survival time from the controls. Both sexes showed a significant dose response in early mortality with no significant departure from linearity ($p<.05$).

The Director concludes from this study that furazolidone is a carcinogen in the mouse as demonstrated by the significant increase in the incidence of mice with bronchial adenocarcinomas and with all malignant tumors combined for males at the 0.015% and 0.03% dose and for females at the 0.03% dose compared with controls, and by the significant linear dose-response relationships for malignancies. He further concludes that furazolidone resulted in early mortality in both the male and female mice.

NORWICH'S HORMONAL HYPOTHESIS

Prior to completion of lifetime studies discussed above, the only direct experimental evidence that furazolidone (NF–180), nitrofurazone (NF–7), nihydrazone (NF–64) and furaltadone (NF–260) are potential carcinogens rested upon the finding of the induction of mammary tumors in female rats. These tumors were the same types as those which are known to occur spontaneously in the aging rat. Male rats had not been affected and there was no increase of non-mammary tumors in females. However, these studies had not involved lifetime exposure to the drugs. In addition to the evidence concerning these four nitrofurans, there was evidence that other nitrofurans, e.g., 2-(2-formylhydrazine)-4-(5-nitro-2-furyl)-thiazole, were tumorigens and that their effects were not limited to the mammary gland of female rats.

In 1969, Norwich began studies on the "Effects of Nitrofurans on Steroidogenesis and Mammary Hyperplasia." Progress reports were periodically submitted to FDA. In response to the 1971 FEDERAL REGISTER notices, Norwich made reference to these studies and claimed that the nitrofurans disrupted the normal hormone function in rats at the very high doses given in the tumorigenic studies, and this disruption abnormally stressed the mammary gland and resulted in the induction at an early age of the types of tumors normally found in very old rats. Norwich further claimed that its methods of analysis for residues of the drug were adequately sensitive to assure the absence of residues well below the level that would cause the hormonal disruption.

Norwich was requested by FDA to compile all available data relating to its hormonal hypothesis and to present it for evaluation by toxicologists from the Bureau of Foods and endocrinologists from the Bureau of Drugs and the National Institutes of Health. On December 10, 1973, Norwich made an oral presentation and submitted all of the relevant material for review. The substance of the hypothesis is as follows:

Norwich claimed that nitrofurans interfere with the normal catabolism of the hormone, progesterone, by the adrenal cortex to the hormone, corticosterone, and that the female Sprague-Dawley rat is particularly sensitive to the drugs' effect. Corticosterone acts on the hypothalamus to inhibit the release of another hormone, ACTH. ACTH is the tropic hormone which stimulates the adrenal gland to produce more progesterone. When the nitrofurans interfere with the catabolism of progesterone to corticosterone, there is less corticosterone to inhibit the hypothalamus and the hypothalamus reacts by secreting more ACTH, which in turn prompts further secretion of progesterone from the adrenal. Its conversion is blocked and because the negative feedback loop is interrupted, there is a resulting increase in circulating plasma progesterone. The progesterone circulates through the body to the mammary gland where it serves its physiologic function of stimulating mammary growth and anatomical differentiation. Norwich suggests that when a rat is placed under the continuous progesterone stimulation by the mechanism described, the mammary gland will respond as if in the first trimester of pregnancy. The result will be repeated surges of rapid cellular division and mammary differentiation with production of more mammary cells in a given time period than would occur in an ordinary rat not under the stress. Therefore, Norwich speculates that the probability is increased that more spontaneous tumors will be produced and will occur earlier in life.

Norwich further contends that in humans, unlike rats, the hormone cortisol is the primary mediator of ACTH release, and the conversion of progesterone to cortisol does not require the intermediate synthesis of corticosterone. Thus, it claims that if the hormonal theory (of Norwich) is correct, the process interfered with by high levels of furazolidone (NF–180) in rats does not even occur in humans.

Norwich suggests that prolactin may also be involved in the tumorigenic response. The pituitary gland may be stimulated because of the projected low plasma corticosterone levels, resulting in an increased pituitary release of prolactin. Therefore, Norwich speculates that both progesterone and prolactin together may induce hyperplasia in the mammary gland favoring the growth of mammary tumors.

In support of this theory, Norwich presented data which it claimed showed that the acute treatment of female Sprague-Dawley rats with furazolidone (NF–180) resulted in an abrupt rise in circulating plasma progesterone. Other data were presented which showed that furazolidone (NF–180) caused an increase in ova in the ovaries of rats and resulted in sexual maturity at an earlier age than would normally occur. Other data showed the induction of an estrus cycle in estrogen-primed rats who had had their ovaries removed. Norwich presented data on the study of adrenal homogenates under in vitro conditions and the effect of furazolidone (NF–180); it compared in vitro the ability of normal adrenals taken from untreated Sprague-Dawley and Fischer rats to utilize progesterone in the synthesis of corticosterone. These two rat strains were used for the comparison because they usually exhibit different spontaneous mammary tumor rates. The normal spontaneous mammary tumor incidence in the Fischer female rat is usually lower than that in the Sprague-Dawley female rat.

Norwich claimed these in vitro studies showed that furazolidone (NF–180) inhibited the synthesis of corticosterone from progesterone. Data were presented to show that differences in furazolidone-

treated Fischer rats and Sprague-Dawley rats in the utilization of progesterone paralleled difference in tumor growth.

Norwich claims its studies showed that furazolidone inhibited the synthesis of corticosterone and enhanced the conversion of progesterone to a pool of unidentified steroids. Norwich claims that deoxycorticosterone was the steroid produced by the adrenals from the furazolidone-treated rats. It also claimed there was an increase of an androstene-type steroid in the profile of urinary steroids. Biochemical studies were also submitted in an attempt to show that the nitrofurans block the 11-hydroxylation step in corticosterone synthesis.

Results with nitrofurazone (NF–7) in adrenals in vitro were claimed by Norwich to duplicate those with furazolidone (NF–180).

Food and Drug Administration scientists have rejected the data presented by Norwich to support the hormonal hypothesis for the following reasons:

No direct evidence was presented to show that furazolidone inhibited the synthesis of corticosterone from progesterone. If the hypothesis were correct, then adrenal venous progesterone or adrenal content of progesterone should be increased and corticosterone diminished. These were not examined. The in vitro studies as conducted are not considered adequate to demonstrate inhibition of the biosynthesis of corticosterone.

The in vitro studies only show that there is an apparent inhibition of the synthesis of a compound with a thin-layer chromatography mobility similar to corticosterone. No data were presented to show that the compound was corticosterone. The report states that the identification is totally dependent on R_f (retardation factor) values which are not sufficiently accurate for definitive identification of hydroxylated 4-pregnene metabolites or their various acetylated derivatives.

Furthermore, if corticosterone synthesis were inhibited the adrenal cortex would be expected to increase in weight. However, no adrenal weight change of treated versus control rats was evident.

The Norwich claim that the 11-hydroxylation is inhibited is supported by scanty and inadequate data, and the data on the profiles of the urinary steroids (17-ketosteroids) are essentially meaningless. They include gas chromatography of unpurified urinary extracts which are uninterpretable. Variations in the profile under such experimental conditions may be due to a multitude of factors: impurities, breakdown products formed during hydrolysis whether of steroidal origin or not, etc. No data on characterization of deoxycorticosterone are presented. The report states that the material was identified by purification through various chromatographic procedures. Chromatography is not considered adequate for structural identification of "unknowns."

Norwich's data on progesterone levels in plasma are not acceptable. The fluorescence method used is antiquated and not sufficiently specific. The competitive protein binding method used is considered unreliable, and the experimental data on the radioimmunoassay procedure are inadequate.

Although generally the radioimmunoassay procedure is acceptable, the standard curve presented in the report casts doubt that the progesterone measurements in this case are meaningful.

Although prolactin has been implicated in the development of mammary tumors, no direct evidence has been presented by Norwich to demonstrate that furazolidone affects prolactin levels. Stimulation of ACTH secretion may occur without concomitant stimulation of prolactin secretion. Prolactin was not measured, and no evidence was offered by Norwich other than an incorrect supposition to support the concept of increased prolactin secretion.

The role of progesterone in increased mammary cell proliferation involving a rise in mitotic activity is portrayed by Norwich as an oversimplification of very complex mammary physiology, and Norwich incorrectly states that an increase in progesterone stimulates pseudopregnancy in the rat.

From the data presented, the Director concludes that it is not possible to tell how furazolidone (NF–180) affects or accelerates the appearance of mammary tumors. To determine if the hypothesis were operative, ovaryectomized or adrenalectomized rats maintained on estrogen and corticosterone should have been tested to see if furazolidone (NF–180) under these conditions failed to induce mammary tumors.

Even if a hormonal effect had been adequately demonstrated and established as the sole operant in the development of mammary tumors in the female rats studied, the mechanism would have to be sufficiently defined. Then, if the mechanism in rodents was not applicable to humans, it would be necessary to determine that the nitrofurans would not cause a different hormonal effect in humans by using an appropriate animal species to establish a "no-hormonal-effect" level.

More important, the Director concludes that the new evidence of carcinogenicity in the lifetime studies of furazolidone-treated rats and mice is contrary to acceptance of any hormonal hypothesis as solely operant. In these latter studies which are discussed in this section of this notice, the induction of tumors is not limited to effects on the mammary gland of female rats. A variety of tumors are seen in both male and female rats in both strains and include, for example, lymphatic tumors, pituitary and thyroid tumors, skin tumors, and mesothelioma in the scrotal cavity of Fischer male rats. In the mouse study lung cancers were the most prevalent, with no sex related differences in the incidence of tumors. There was no significant drug effect on the incidence of nonmalignant or malignant tumors in endocrine tissues of these mice.

The Director therefore concludes that the hormonal hypothesis is not relevant to the finding that furazolidone is a carcinogen.

However, the following facts are significant in regard to the tumor-inducing properties of furazolidone as demonstrated in the four chronic studies:

1. Furazolidone-induced malignancies in both rats and mice.

2. Furazolidone also induced nonmalignant tumors.

3. In most cases the induced malignant and nonmalignant tumors were found at the same sites.

4. There is some evidence that the induction of benign tumors is also related to the induction or progression to malignancies as a function of dose.

5. There are marked sex differences in the tumor responses in the rats. There are also some commonalities in the tumor sites of the male and female rats.

6. Mammary tumors were the predominant effect in the female rats.

7. Nonmammary tumors were induced in the Sprague-Dawley male rats with no one type being singularly significant. However, in the Fischer rats, several specific nonmammary tumors were induced in both male and female rats.

8. The major target site in the mouse was the lung. There was no evidence of a significant difference between male and female mice in tumor response and tumorigenic effects in endocrine tissues.

9. The induction of tumors involved not only increased incidence of animals with tumors but increased numbers of tumors per animal.

10. There is no reason not to accept the appropriateness of the rats and mice as a bioassay from which to predict the cancer potential in humans. No relevant data in humans are available.

From these facts, the Director concludes that furazolidone induces cancer in both rats and mice, and therefore its use as a drug in food-producing animals is subject to the requirements of section 512 (d) (1) (H) of the Federal Food, Drug, and Cosmetic Act. The sum total of evidence indicates that furazolidone induces both site-specific tumor effects as well as an overall effect on random tumor induction; there is clear evidence of malignancy and a concurrent induction of benign tumors, the proportions of which increase in favor of malignancies with an increase in dose and probably also involve a relationship of latency and dose; and that there is an adverse effect on mortality which may bear a relationship to the tumors. The accumulation of these facts requires that the evaluation of the total neoplastic activity of the carcinogenic agent, furazolidone, include consideration of all tumor induction as potentially significant to human health. Since the susceptibility in humans is not defined, furazolidone must be assumed as a potential human carcinogen and safeguards taken to assure no human exposure. Therefore, a conservative approach to the estimation of a theoretical "no-effect" level of exposure in humans for purposes of defining the minimum required assay sensitivity for residues is required.

II. ADEQUATE, RELIABLE AND PRACTICABLE METHODS OF ANALYSIS ARE NOT AVAILABLE FOR MONITORING FOOD NOR CAN CONDITIONS OF USE BE SPECIFIED IN THE LABELING TO ASSURE THAT THERE ARE NO RESIDUES ATTRIBUTABLE TO THE USE OF FURAZOLIDONE

The Director has concluded that furazolidone has been shown to induce cancer when ingested by animals. Therefore its continued approval for use in animals depends upon the sponsor's showing that the use of the drug will not adversely affect the animals for which it is intended and that no residue of the drug will be found in any edible portion of the animals after slaughter or in any food yielded by or derived from the living animals. To make this showing, a multiple step process is necessary.

First, the amount of total residue in the edible tissues and the depletion of residues with time must be determined.

Second, sufficient information must be developed about the identity of the residues and their proportionate amount to the total residue to determine if any metabolites need to be tested in carcinogenic bioassays in addition to the parent drug.

Third, from the carcinogenic bioassay data, a theoretical statistical estimate must be determined for the maximum concentration of total residues of possible carcinogenic concern that can be present in the total daily diet of humans that can be considered insignificant and to pose essentially no risk of cancer to humans.

Fourth, sufficient information about the residues must be developed to: (a) Determine the tissue (called the target tissue) from which residues deplete most slowly and must be monitored by the regulatory assay, and (b) determine the compound that is to be measured in the regulatory assay so that its measurement can assure there will be no residues of potential carcinogenic concern. (This compound is called the marker.)

Fifth, a practicable method of analysis must be developed for residues that can measure the marker in the target tissue at a concentration level which at least assures that the total residues of potential carcinogenic concern in the total daily diet of humans does not exceed the level established in step three above.

Sixth, it must be demonstrated that practical conditions of use can be prescribed on the label of the product to assure "no residue".

Each of the steps involves principles which are essential for a determination of food safety and compliance with the requirements of the act to assure "no residue" in human food from animals treated with a carcinogenic drug. Each step builds upon the preceding step. Therefore, failure to accomplish any one of the six steps in whole or in part for any drug that is a carcinogen ordinarily will preclude a sponsor's fulfilling its obligation under the act.

The sponsors of furazolidone have failed to meet the requirements of the first step. On this basis alone, they cannot meet the statutory requirement of showing "no residue." Nor can they proceed with the fulfillment of all of the remaining steps. The deficiencies in each of the steps are discussed in detail in the following subsections:

(1) *The Amount of Total Residue in the Edible Tissues and the Depletion of Residues with Time Has Not Been Determined.* When a drug is administered to an animal, the drug may be altered by the animal into a variety of compounds called metabolites. The process of alteration may be such that little residue of the parent drug remains in the animal tissue, but there may be considerable metabolite residue. Therefore, the total residue with which FDA is concerned includes both the parent drug and the metabolites.

The composition and amount of each of the residues depend upon the metabolism in a particular species. Because there are species differences in metabolism, it is necessary to determine metabolism and residues in each of the species for which the drug is used. Thus § 514.1 (b) (7) (i) (21 CFR 514.1(b) (7) (i)) includes metabolism and residue depletion studies among those kinds of studies which are used to determine residues.

Exposure to furazolidone in the diet induced cancer in rats and mice. It is not known if the parent drug was the carcinogenic agent or if the carcinogenic response was due to one or more active metabolites. Such lack of knowledge is not unique to furazolidone; it is typical of most cancer-inducing agents that the mechanism of the induction of cancer or the identification of the carcinogenic agent is not known. Therefore, when considering the safety of food from furazolidone-treated animals, all drug-related residues to which humans may be exposed must be assumed to be potential carcinogens unless convincing evidence is presented to the contrary. Such evidence consists of not only the identification of the residues that are claimed to be safe, but also adequate toxicological studies showing that the levels of each of the residues claimed to be safe are in fact safe. All residues not identified or not shown to be safe must be considered potentially carcinogenic and must be required to be below a level of detection that would pose no risk of cancer in humans.

The use of chemical assays to determine initially the total residues requires knowledge about what residues occur (parent drug and metabolites) and requires the availability of adequate methods of analysis to measure each one of the residues at the level of interest. Therefore, this chemical approach is usually not feasible. Instead, a radiolabeled drug is used to provide a practical and adequate approach to the initial measurement of total residues in the edible tissues. In this approach, the drug is synthesized with a radioisotope so that when the drug is administered to the animal according to label directions, all resulting residues of interest can be measured in the edible products. This is accomplished by counting the radioactivity in the tissues and converting these measurements to an estimation of the amount of residue expressed as parent drug equivalents. The conversion is made based on the known specific activity of the radiolabeled drug used in the study.

Although a number of reports have been submitted on the quantity of furazolidone in the edible tissues of drug-treated animals which will be discussed in step (6), no data have been submitted by the sponsors on total drug residues in edible tissues from furazolidone-treated animals.

On July 23, 1971, Norwich submitted a report, "NF-180 Residues in Chicken Tissues," Project No. 576.03, dated July 23, 1969, which mentions two metabolites that may occur as residues of furazolidone (NF-180) in edible tissues. The report gives no information on the identity or quantity of the residues or any indication of the evidence on which the presumption is based. The Director assumes the compounds are those designated by Norwich as NF-362 and NF-682 in a letter to FDA on October 1, 1969, where it was stated that methods were being developed for their analysis.

Data on the metabolism of other nitrofurans has been submitted by Norwich which, however, cannot be accepted as a substitute for metabolism data on furazolidone in the animals of intended use because the metabolism of each compound may differ in each of the animal species.

Because no relevant data have been submitted on metabolism and residue depletion of furazolidone, the Director concludes that the amount of total residue in in the edible tissues and the depletion of residues with time has not been determined.

(2) *Sufficient Information Has Not Been Developed About the Identity of the Residues and Their Proportionate Amount to the Total Residue to Determine if any Metabolites Need to be Tested in Carcinogenic Bioassays in Addition to the Parent Drug.* The composition of a drug's residue may vary from the extremes of less than 1% parent drug and more than 99% metabolites to less than 1% metabolites and more than 99% parent drug, although the latter case would be rare. The Food and Drug Administration must estimate the carcinogenic potency of the total residue because this is what man will be exposed to in food. Ideally this estimation would be based on the identification of each of the metabolites in the residue, their synthesis, and subsequent testing of the parent drug and all of the metabolites for carcinogenicity. However, this approach is usually impractical.

Various approaches are available to limit the number of residue compounds for which carcinogenic bioassays are required to provide a sufficiently conservative estimate of the carcinogenic potency of the total residue. However, Norwich has not presented any quantitative or qualitative information about the total residues of furazolidone in edible tissues.

Therefore, the Director concludes that there is insufficient information for FDA to determine if the carcinogenic testing

of furazolidone is adequate to estimate the carcinogenic potency of the total residue and to determine if a "safe" dose, described in step (3), based on the carcinogenic testing of furazolidone is adequate to protect the public health.

(3) *A Theoretical Statistical Estimate for the Maximum Concentration of Total Residues of Possible Carcinogenic Concern That Can Be Present in the Total Daily Diet of Humans That Can Be Considered Insignificant and to Pose Essentially No Risk of Cancer to Humans Has Not Been Determined.* The exception to the Delaney Anti-Cancer Clause was enacted to permit the use of a carcinogenic drug for food-producing animals, if, among other requirements, the use does not result in residues in foods as determined by a method of analysis prescribed or approved by the Secretary. The enactment of this provision followed lengthy testimony and debate on the use of diethylstilbestrol (DES) for increased feed efficiency in cattle.

During the debates, testimony was presented on the mouse uterine assay used for the detection of DES residues in animal tissues. It was argued that by this highly sensitive technique, which could measure DES as low as 2 ppb, no residues of DES would be found in cattle treated with the drug when an adequate drug withdrawal period prior to slaughter was observed. In 1962, the capability to detect 2 ppb of any residue was an unusual achievement for assay methodology and was then regarded as a reasonable definition of "no residue".

Because the act leaves the choice of an assay method to assure "no residue" for any specific carcinogenic animal drug to the judgment of the Secretary, an operational definition of "no residue" had to be adopted. Because there was no way to be sure that the best available analytical method would always be sufficiently sensitive and practicable to provide adequate protection against cancer, FDA customarily required a lower limit of reliable measurement of at least 2 ppb for carcinogens. This level was based primarily upon the judgment of scientists that the absence of residues at 2 ppb or above usually would assure that any remaining residue was biologically insignificant.

Since 1962, technological advances in research methods, e.g., the advent of immuno-assay techniques, advances in ultrasensitive mass spectrometry, and the use of radiotracer techniques in metabolism studies, now open the possibility that residues at concentrations considerably below 2 ppb may be found.

Because of these developments, FDA has been examining procedures which might be used to establish the required lower limit of reliable measurement for residue assay methods. Currently, FDA will accept the use of conservative statistical extrapolation procedures that relate the carcinogenic potency of the drug to the determination of the minimum requirement for residue assay sensitivity. However, if a more sensitive practicable method for the determination of residues is available, FDA will adopt that more sensitive method.

A proposed regulation on this matter was published in the FEDERAL REGISTER of July 19, 1973 (38 FR 19226). Numerous comments were received and have been considered. Based on the review, FDA has tentatively concluded that a modified version of the approach described in the proposal offers a sound approach to this extremely complex issue. Efforts are underway to establish by regulation the use of the new procedure for determining the minimum required sensitivity for the tissue residue assay for carcinogenic drugs.

On November 11, 1975, Norwich made a submission to FDA that included a report prepared by Mr. Nathan Mantel estimating the "safe" dose for furazolidone. The "safe" dose (S_e is a theoretical statistical estimate which represents the maximum concentration of the residues in the total daily diet of humans which could be considered insignificant and pose no risk of cancer and is thus "safe" within the meaning of the act. The S_e was derived from use of the data from the four chronic studies on furazolidone, discussed in section I of this notice, and the application of the Mantel-Bryan extrapolation model. (N. Mantel and W. R. Bryan, "Safety Testing of Carcinogenic Agents," Journal of the National Cancer Institute, 27:455–470 (1961). N. Mantel, N. Bohidar, C. Brown, J. Ciminera and J. Tukey, "An Improved Mantel-Bryan Procedure for Safety Testing of Carcinogens," Cancer Research, 35:865–872 (1975).) The model was used to estimate S_e based on a probability of the occurrence of cancer of 1 in 1 million with a slope of 1 probit unit per log increase in dose at the 99% confidence level. Norwich concludes that 21 ppb is the estimated S_e for furazolidone. The calculated "safe" concentrations of furazolidone in food were determined by Norwich to be 63 ppb in muscle, 21 ppb in milk, and 63 ppb in eggs using the food consumption adjustments proposed in a paper presented at the Gordon Research Conference on Drug Metabolism, June 23–27, 1975, by two FDA scientists, M. K. Perez and N. E. Weber, entitled "Requirements for Metabolic Studies—Impact on Food Safety Regulation." Norwich claims that the regulatory methods it has proposed for control of residues are of adequate sensitivity to assure the absence of residues above 2 ppb and are certainly adequate to assure the absence of residues at levels as high as 21 and 63 ppb. Therefore, it contends, it has demonstrated the safe use of furazolidone. For this reason, it is necessary for the Director to consider whether or not the sensitivity required for the assay as proposed by the sponsors is adequate to assure safe use of the drug.

As shown in step (2), adequate information has not been provided to determine if an S_e can be established based on the carcinogenicity testing of furazolidone. However, even if such information had been provided and it was adequate to show that the S_e for total residues could be based solely on the furazolidone testing data, the sponsors, as will be shown in this and subsequent sections, have not satisfied the requirements of the act to demonstate the safe use of furazolidone.

Norwich has provided data on furazolidone from which an S_e might be determined, and FDA accepts the basic principles of the Mantel-Bryan model to estimate S_e for a carcinogenic animal drug. However, the way Norwich has selected and used the data in the extrapolative procedure to derive the value of 21 ppb as the S_e for furazolidone is not acceptable. There are two major objections to the Norwich approach. First, Norwich has not used data which are based on all tumors present and diagnosed from histopathological examination at sacrifice. Second, Norwich has based the estimation of S_e on the analysis of only tumors which were malignant.

First Major Area of Objection

Norwich used data which are not based on all tumors present and diagnosed by histopathological examination at sacrifice. It offers the following reason for not doing so. Mr. Mantel objects to the furazolidone chronic study experimental designs, pointing out that for best use of his extrapolative technique, a "near life time" study would have been better.

The Fischer rat study and the higher dose Sprague-Dawley rat study followed protocols where the animals were fed medicated diets for 20 and 18 months respectively, followed by maintenance on a drug-free diet for an additional 10 and 11 months respectively. The experiments were terminated when survival reached approximately 10% The age of the rats at the termination of these studies was in excess of 30 months. Similarly, in the mouse study, the animals received the drug in feed for 13 months and were maintained on a drug-free diet for an additional 10 months. The study was terminated after 23 months when the mice were approximately 25 months old. In contrast the lower level Sprague-Dawley rat study was a 24-month study with continuous drug treatment in the diet. All groups were terminated at 24 months when the rats were approximately 26 months old.

Given the data at hand, Mr. Mantel adjusts the data to fit an experimental design he would have considered better, i.e., an earlier termination. His reasons for the adjustment of the data are to achieve a lower incidence of tumors in the controls; to exclude late-in-life tumors that in his judgment are not significant; and to provide more comparability of the data between the rat studies so that the tumor findings can be combined to provide more values for the estimation of the S_e.

To achieve the above, Mr. Mantel chooses two cutoff times and determines the tumor incidence by the following criteria and assumptions:

1. Tumor findings in all animals that died prior to his "cutoff" time are used based on the histopathology findings.

2. Tumors found in animals that died after the "cutoff" time are counted only if a mass was detected in the live animal prior to the "cutoff" time. If a mass was found in the live animal, histopathology after the "cutoff" time is used as the basis for determining if the mass was a tumor.

3. All animals that were alive at the "cutoff" time and did not have a determinative mass at that time, but were found to have tumors or malignancies at later sacrifice, were not counted.

Mr. Mantel projects the data for "cutoff" at 24 and 22 months for rats and 18 to 16 months for mice. The 24-month rats and the 18-month mice cutoffs represent the time animals were on the experiment, having begun on the experiment at approximately 2 months of age. The second set of figures, 22 months for rats and 16 months for mice, were also used because these times represented what Mr. Mantel considers the lifespan age of the animals (24-month old rats and 18-month old mice). The Kolbye-Perez paper referred to in step (4) is erroneously interpreted and cited as support for this definition of lifespan.

The differences between use of 24 or 22 months for rats and 18 or 16 months for mice are not sufficiently large to make a significant difference in the estimation of an S_0. However, there is a significant difference in the estimated S_0 when either of the "cutoff" times are used rather than the use of all tumor data at sacrifice.

To elaborate on the effect of Mr. Mantel's adjustment of the data, the following example is given. In the mouse experiment, 26 of 48 male mice at the lowest dose had malignancies at termination of the study, i.e., 23 months on experiment. At an 18-month "cutoff" time, only 3 males at the low dose had malignancies based on his criteria. Using Mr. Mantel's approach, it has to be assumed that the 23 male mice deemed tumor-free at 18 months developed all of the malignancies during the last 5 months of life. It also has to be assumed that the tumors or malignancies which developed in the animals late in life are not significant even though the studies showed that the drug-treated groups in some cases had more tumors than the control groups late in life. In other words, the development of tumors or malignancies in the aged, drug-related or not, are not important. This assumption is not acceptable to FDA.

The objections of FDA to Mr. Mantel's approach of using a "cutoff" time for tumor determination are two-fold: (a) The use of palpation as the sole determinant of the presence of tumors in live animals, and (b) the arbitrary determination that tumors observed beyond a certain age are of no consequence.

On the first point it may be argued that palpation followed by histopathological confirmation through biopsy or at sacrifice can be effectively used to study the development of certain types of tumors, e.g., mammary tumors. If systematic examination is conducted by specially trained personnel, it may be possible from the information to plot the development of benign tumors with time and to determine the progression of benign tumors to malignancies. It is not known if an adequate systematic procedure of palpation was followed in the furazolidone studies and it cannot be assumed that if a mammary tumor was present it would be palpable. Palpation may be an aid in deriving additional information which may be helpful in evaluating the data, but it alone cannot be used in the manner proposed by Norwich with the exclusion of some tumor data at sacrifice.

In addition, furazolidone induced tumors at sites other than the mammary gland. Because furazolidone exhibits a random tumor effect in addition to site-specific effects, not limited to the mammary gland, palpation even under the most carefully controlled conditions cannot be presumed adequate to determine the tumor status in live animals in this case. Tumors and malignancies of the type induced by furazolidone can be present in a live animal and not be palpable or otherwise observed until autopsy and histopathology.

During discussions with Norwich concerning studies FDA had requested in support of human drug uses, the Bureau of Veterinary Medicine recommended that Norwich seek guidance on the design of the studies from Dr. Weisberger, who was at that time with the National Cancer Institute. It is assumed that the protocols followed Dr. Weisberger's recommendations, and that Norwich adopted the recommended protocols as an appropriate way to demonstrate the safety of furazolidone. Norwich states in its report that the animals were terminated when 10% survival was reached, except for the low-level Sprague-Dawley rat study where all groups were terminated after 24 months on test.

Scientific opinion on the design of carcinogenic bioassays is continually changing, based on new knowledge and experience. The design of protocols is primarily influenced by the particular questions being asked. Presumably, some of the questions that the furazolidone studies addressed included determining whether or not furazolidone was a carcinogen; whether the only tumor effect would be in the mammary gland of female rats through hormonal disruption that is unique to the rat and not applicable to humans; and whether at lower levels of exposure there would be no tumorigenic risk. These were reasonable questions from Norwich's viewpoint considering the available evidence at that time and the opinion of the Norwich scientists that this course of action would support the safe use of furazolidone. When the furazolidone studies were initiated, there was no reason to give any thought to the possible use of the data in an extrapolation model, such as the Mantel-Bryan model, to estimate S_0.

It is also evident that, although a high spontaneous tumor rate in Sprague-Dawley rats may have been anticipated, it was not expected that the high spontaneous tumor rate found in the Fischer rats would occur. The anticipated differences in the spontaneous tumor rate for the two rat strains, which were not borne out by the data, served as one of the basic reasons why both strains were chosen.

Protocols now recommended by FDA for chronic toxicity-carcinogenicity testing are different from the protocols used for the carcinogenicity testing of furazolidone. These current protocols include the recommendation that drug treatment be continuous throughout the experiment and that sacrifice times for the various groups be determined by criteria different from those used in the furazolidone studies. The agency does not disagree with Mr. Mantel's opinion that the design of the experiments and the resulting data are not ideal for use of the extrapolation model to estimate S_0 dose. However, this has no bearing on the fact that the data from the four chronic toxicity studies of furazolidone are the best that are available on the carcinogenic potency of the drug. Thus, FDA is obligated to make decisions based on the data at hand, using the best techniques available for this purpose, although both the data and the extrapolative techniques have limitations.

To address the second point, FDA cannot accept an arbitrarily determined lifespan for test animals and the assumption that tumors beyond a certain age are insignificant. For rats, it is true that the increase in tumors with age tends to plateau at about 24 months of age. However, total tumors in a rate population may continue to increase slowly. Unless a regression mechanism is operative in the late-in-life period, any tumors seen in rats at ages beyond 24 months would include those produced up to 24 months of age plus any tumors produced after this period.

In the furazolidone studies there were significant increases in the numbers of treated rats and mice with tumors (benign and malignant combined) when tumors were counted as a cumulative index at the time of sacrifice in comparison with controls, in spite of the high spontaneous rates seen in the control groups.

The significance of these data bear on the human safety determination. Cancer is generally a disease of the aged. As man's life expectancy increases due primarily to modern medicine and improved nutrition and environment, the probability that cancer will cause death also increases. The expression of cancer usually evolves after a period of latency. The dose level, frequency, and duration of exposure to a compound are related not only to whether cancer is induced but also to the latency period before the expression of cancer. Other important factors may operate to accelerate the expression of cancer.

It is therefore important, when using test animals to determine if a compound may pose a carcinogenic risk to man, to consider the effects during the various stages of life including the late-in-life periods.

It is important to consider that the strong carcinogens and proximate carcinogens are generally more easily identified through testing in carcinogenic bioassays than are the weak carcinogens and the procarcinogens (a compound that becomes altered through metabolism to form an active carcinogen), cocarcinogens, tumor promotors and tumor accelerators. It is likely that the number of weak carcinogens and nonproximate

carcinogens exceeds the number of strong carcinogens and proximate carcinogens and would represent the greater number of compounds for which FDA would require carcinogenic testing if their possible presence in food was under consideration. The duration of the study may affect whether a cancer-inducing effect is detected. Whether a compound is a proximate carcinogen or a procarcinogen of whether the compound is a cocarcinogen or acts to induce or accelerate the occurrence of tumors which occur in the aging process is immaterial. If the end result of exposure to the compound results in a shortening of life, the significance to man is great.

It is therefore important, in testing for carcinogenesis, to determine as many effects as possible throughout the lifespan. The furazolidone data indicate a significant drug-related effect on mortality. The data also show a significant drug effect on the onset time of tumor development, tumor multiplicity, and as stated previously, an effect on the cumulative number of tumors throughout life, in spite of the high spontaneous rate in controls.

Mr. Mantel states that his extrapolation model provides a best estimate of S_0 dose when the incidence of spontaneous tumors is low. This is one of his major reasons for using a "cutoff" period and using only malignant tumors. He states that his procedure is much more rigorous and conservative when the incidence of spontaneous tumors is high, and he further states it is more difficult to determine experimentally a drug-induced effect when these conditions prevail. For the reasons previously stated, FDA does not accept that these factors warrant adjustment of data to achieve artifically a low control incidence. In spite of the difficulties in detecting a drug-induced effect when the tumors are of the same type as those which occur naturally, such effects were seen for furazolidone, based on histopathology at sacrifice, and cannot be dismissed.

The fact that the extrapolation model provides a more conservative estimate of S_0 when the spontaneous tumor rate is high is not an inconsistency in the model per se. It is due to increased variability in estimating induced tumor rates over the high spontaneous rate in controls. When a nature of the compound's carcinogenic potential is such that measurement of that potential is more difficult in a bioassay with small sample size, it is not unreasonable that the model treat this situation in a conservative fashion.

SECOND MAJOR AREA OF OBJECTION

Norwich based the estimate of "safe" dose on the analysis of malignant tumors only. The firm explained that it did so because malignancy is the major sign of carcinogenesis; a nonmalignant tumor occurring late in life has little opportunity to transform to malignancy, except perhaps very late in life; and a reduced spontaneous rate is achieved when only malignancies are used.

Norwich has also stated that nonmalignant tumors in long duration studies have such a high rate of spontaneous appearances as to make unfeasible the establishment of "safe" levels by the Mantel-Bryan approach. Norwich states that in the rate experiments, unrealistically low "safe" levels are derived primarily because, with the high spontaneous tumor occurrence, the Mantel-Bryan procedure is unable to distinguish the difference between the induced rate and the spontaneous rate.

Although there is no disagreement that malignancy is the major sign of carcinogenesis, FDA objects to the Norwich approach because in evaluating tumor data, several situations may arise:

1. The compound induces malignant tumors;

2. The compound induces both malignant and benign tumors;

3. The compound induces only benign tumors:

(a) which experts might agree pose no risk of cancer and for which criteria other than a Mantel-Bryan extrapolation are more appropriate to estimate S_0, or

(b) which experts judge are equivocal for interpretations about cancer potential and should be treated as carcinogens until any uncertainties are resolved.

Special consideration is given to compounds that fall into situation 3(a) above. However, for those compounds in the remaining situations, a conservative approach must be taken in the estimation of S_0 to assure adequate protection of humans from exposure to carcinogens and suspect carcinogens. The gray zones of interpretation of tumor findings are recognized along with the limitations in the state-of-the-art for carcinogenic bioassay. Therefore, both malignant and benign tumors combined ordinarily should be used for the extrapolation of S_0. Certain tumors would be excluded only when there was a risk that these tumors, if included, might dilute the estimate of the total neoplastic activity.

When a compound is known to induce malignant tumors, the concurrent induction of benign tumors has a greater weight of importance in assessing neoplastic activity than might be given in the absence of evidence of malignant tumors. The fact that organ specificity has been demonstrated does not allow one to place any less significance upon the cumulative induction of tumors at all sites.

As presented in the 10 summary points at the conclusion of section I of this notice, there was evidence of the induction of malignancies in both species; of different target sites in rats and mice; and of site-specific and random-tumor induction in both species. When these findings are combined with the fact that there is an absence of information on human sensitivity to furazolidone, it must be assumed that all tumors (malignant and benign) have significance in assessing the neoplastic activity of furazolidone to assure adequate protection of public health.

Limitations in the state-of-the-art to evaluate cancer potential must also be weighed in judging the degree of conservatism which must be applied. Animal experimentation has its limitations as a means of estimating the neoplastic potential for humans. Major considerations include the limitations in the test animal group size, and susceptibility of the test animals compared to the unknown susceptibilities of a heterogeneous human population. Also, humans are exposed to an array of stress phenomena, including exposure to many agents which may be cocarcinogens.

There are also uncertainties and limitations in the examination of some tumors to determine if they have malignant potential. For example, in the early stages of a mammary tumor growth, the mass may be chiefly adenomatous. As growth continues, the supporting white fibrous connective tissue may also begin to proliferate, resulting in a fibroadenoma. Later, the adenomatous part of the neoplastic mass may entirely disappear, leaving a fibroma. Finally, a part of the adenoma or fibroadenoma may develop malignant tendencies and that portion may then become an adenocarcinoma. All four tumor types, i.e., adenoma, fibroadenoma, fibroma, and adenocarcinoma may be present in the same tumor mass. A small focus of adenocarcinoma in the early stage of a malignant tumor may be overlooked in the tumor growth unless adequate multiple sections of the tumor are made.

Also, unless great care is exercised in the evaluation of the spread of an adenocarcinoma of the mammary gland, the metastasis along the lymphatic rout passing through, under or near other mammary glands may be considered to be separate primary adenocarcinomas rather than a single primary growth with multiple metastases.

It may be debated whether fibroadenomas of the mammary gland in humans progress to adenocarcinomas. It is difficult at best to interpret inferential information from human experience because adequate controlled studies in humans are generally not feasible. Regardless of what is known about mammary tumors in humans, it cannot be assumed that furazolidone-induced mammary tumors in rats are comparable. Furazolidone may cause different kinds of tumors (type and site) in man than in rats, and it may influence not only the induction of benign tumors in man but also the progression of benign tumors to malignancies.

Furazolidone is not limited to effects on the mammary glands. For the various other types of tumors, such as the lung tumors in the mice, it is precarious to assume, for example, that bronchial adenomas are not precancerous.

Demonstration that furazolidone induces malignant as well as benign tumors dictates the need to follow a conservative approach and to consider all tumors in the evaluation of the neoplastic potency for furazolidone and the estimate of S_0.

ANOTHER OBJECTION TO THE NORWICH APPROACH

Norwich combined the male and female rat tumor data and the data from all of the rat studies in the estimation of

S_0 in the rats. When the Norwich approach and assumptions described and rejected in the preceding discussion are used, there is no statistically significant difference between the sexes based on the incidence of rats with malignancies at the "cutoff" period. Mr. Mantel admits in the Norwich submission that the protocols of the three rat studies are not of compatible design for pooling the data; however, he then makes the adjustments described above in order to be able to pool the data.

The combining of the male and female rat data is not acceptable to FDA because the tumor types are sex-related. In each of the experiments the predominant effect seen in the female groups was a mammary tumor effect, although other tumor types also were observed. However, in each of the male groups, no significant mammary tumor effect was observed, and significant effects were observed for other tumor types.

Also, FDA does not accept the pooling of the data in the three rat studies because of the differences in the protocols used and because FDA does not accept the adjustment of the data as proposed by Norwich to provide more comparability among the studies. However, FDA does accept the combination of male and female mice data because there are no significant differences in the tumor responses of the sexes.

According to the Norwich approach, the mouse data (male and female combined) provided the most conservative estimation of S_0, i.e., 21 ppb. The Director concludes that this estimated "safe" dose of 21 ppb for furazolidone is not acceptable because of the way in which Norwich selected and used the data. The Director observes that if the data provided by Norwich are correctly used in the extrapolation procedure proposed by Norwich, the S_0 estimate for furazolidone would be 0.5 ppb based on the female rats in the low-dose Sprague-Dawley study and the female rats in the Fischer study, each separately considered.

However, the Director concludes from the preceding step that adequate information has not been presented to show that the estimate of S_0 for furazolidone can be used as an adequately conservative estimate of S_0 for total residue.

Even if it is assumed that the S_0 of 0.5 ppb is adequate, the sponsors of furazolidone have failed to provide adequate methods of analysis to assure no residues. For example, the daily human consumption of muscle tissue might be one-third of the total daily diet excluding fluids based on that segment of the population which consumes meat as a high proportion of the diet.

Therefore, to assure adequate protection of the public health, it would be necessary for the sponsors at least to show that total residues in muscle tissue did not exceed 1.5 ppb in order to assure that the human exposure in the total daily diet does not exceed 0.5 ppb. As demonstrated in step (1), Norwich has not provided this showing. In addition, the succeeding steps show that the available methods of analysis are not adequate. Nor can conditions of safe use be established.

(4) *Sufficient Information Has Not Been Developed about the Residues to Determine the Tissue from which Residues Deplete the Slowest and Must be Monitored by the Regulatory Assay (Called the Target Tissue) and the Compound that is to be Measured in the Regulatory Assay so that its Measurement Can Assure There Will Be No Residues of Potential Carcinogenic Concern. (This Compound is Called the Marker.)* In the Norwich submission of November 11, 1975, reference is made to the Perez-Weber paper, cited above, and another paper by FDA scientists A. C. Kolbye and M. K. Perez entitled "Human Safety Considerations from the Use of Anabolic Agents in Food-Producing Animals" (Coulston/Korte Environmental Quality and Safety, Supplement V. in press—George Thieme Verlag Stuttgart). Copies of these papers have been placed on file with the Hearing Clerk, Food and Drug Administration. These papers describe the basic approach accepted by the agency for use of the extrapolative model in estimating S_0 and then proceeding from S_0 to the estimated "safe" concentration in edible products and finally proceeding to the derivation of the minimum required assay sensitivity.

However, the Norwich submission ignores these basic steps and the information required to derive the minimum required assay sensitivity.

(i) Determination of the Target Tissue: The most practical approach to monitoring and controlling residues in the field is dependent on the selection of that edible tissue from which residues deplete to a "safe concentration" the slowest. Therefore, by monitoring this tissue to assure that residues are below a "safe concentration," it can be assured that all residues in the carcass are below "safe concentrations." In lieu of this determination, all edible tissues must be monitored—placing an additional burden on public funds for monitoring.

The target tissue is readily identified if the requirements of step (1) are met. However, Norwich has failed to meet these requirements and therefore a target tissue cannot be selected.

(ii) Determination of the Compound (Marker) which can be Measured by the Analytical Method to Assure Control of Total Residues: Because the radiolabeled drug approach to the measurement of total residues is not applicable to field use, an alternative approach must be developed to assure control of total residues. This is achieved by selecting an appropriate residue compound, which may be the parent drug or one or more metabolites, in such a way that its measurement serves as a valid indicator that when there is no residue of the marker in the target tissue, there are "no residues" of carcinogenic concern. This step is essential to determine if the method of analysis for regulatory use is adequate. To determine a valid marker, adequate information, such as is derived from conducting an adequate metabolism study, is required which relates the proportionate amount of each of the residues to the total residue and their change in quantitative relationships with time. The marker must be a known proportion of the total residue when the total residue depletes to the "safe concentration" in the target tissue. It also must be structurally identified so that an adequate and specific method of analysis can be developed for its determination.

The regulatory method proposed by Norwich to control residues uses the parent drug, furazolidone, as a marker. Norwich has failed to submit substantiating evidence that the measurement of furazolidone is adequate to control total residues of potential carcinogenic concern.

Norwich has failed to provide data on the amount of total residues and to determine the quantitative relationship of the parent drug to the total residue to see if it can serve as a valid marker as described in the Perez-Weber paper cited previously and referred to by Norwich. If it were assumed that the parent drug could serve as a valid marker, its amount in tissue when total residues reach the "safe concentration" in tissue would have to be known as an essential figure from which to compute the minimum required assay sensitivity. Norwich has failed to provide any of this information.

Even if FDA were to assume that 1.5 ppb is the "safe concentration" in the edible tissue; and it were to assume that furazolidone is 90% of the residue (which is very unlikely); and if FDA were to further assume that a method of analysis were available that would reliably recover 75% of the residue from tissue, which is not the case as shown in step (5), then, even with all of these assumptions, the method of analysis would have to have as a minimum a lower limit of reliable measurement of about 1 ppb to assure control of total residues of potential carcinogenic concern. As also shown in step (5), the available methods of analysis do not even meet this sensitivity, based on very liberal assumptions.

The Director concludes that the essential information required to determine if the measurement of furazolidone can be used to control total residue, within the meaning of the act, has not been provided. Although sufficient information has been provided for the Director to conclude that the current methods of analysis are not adequate, the Director cannot prescribe those conditions that would be adequate because of the lack of essential information.

(5) *A Practicable Method of Analysis Has Not Been Developed for Residues That Can Measure the Marker in the Target Tissue at a Level Which at Least Assures That the Total Residues of Potential Carcinogenic Concern in the Total Daily Diet of Humans Does Not Exceed the Level Established in Step (3).* Section 514.1(b)(7) (21 CFR 514.1(b)(7)) requires the sponsor of a new animal drug application to provide a practicable method of analysis to be used for the control of residues of his product. When the drug in question is a carcinogen, it is also required by this section

that the method be presented in a form suitable for publication in the FEDERAL REGISTER. A "practicable" method is defined by FDA as one which reliably measures the compound throughout all residue levels of interest, and also is sufficiently rapid and reasonable in terms of the expertise and equipment required to be applicable for use in the surveillance and control of residues. "Reliability" is a combination of accuracy, precision and specificity. Accuracy and precision are quantitative terms. "Accuracy" is the measure of deviation from the true value, and "precision" refers to the variability of measurements in replications either by one analyst or within a laboratory or between laboratories. A method may provide reliable measurement if the measurements are not too variable even though it measures less than or more than 100% of the true value. Therefore, accuracy and precision must be considered together.

"Specificity" is a qualitative term. The measurements obtained by the method must reflect only the compound of interest. Because the method will be used on samples from animals for which exposure history is unknown, the techniques used in the method must be adequate to assure not only the integrity of the measurements, but also the identity of the residue to the exclusion of other possible interfering substances. Therefore, when the quantitative measurements are made by techniques which are not inherently specific, e.g., chromatographic techniques, a special confirmatory test is required as part of the official method to assure the identity of the residue.

MEASUREMENT OF THE COMPOUND

Prior to receiving reports that the nitrofurans induced tumors in female rats, FDA had accepted methods for residue analysis supplied by the drug sponsors with a lower limit of detection of furazolidone of 0.1 part per million (ppm) in swine tissue and 1.0 ppm in poultry tissue. These methods were accepted on the basis of validation data presented by the sponsors and were not subject to trial in government laboratories.

After FDA received reports that nitrofurans had induced tumors in female rats, the drug sponsors were advised in 1969 and officially informed in a conference held January 4, 1971, that the residue methods were no longer deemed adequate and that tissue residue methods for furazolidone were needed to measure residues at least as low as 2 ppb. In response to the notices of opportunity for hearing published in the FEDERAL REGISTER in 1971, Norwich and other sponsors submitted newly developed tissue residue methods for furazolidone to FDA for evaluation. The first submission was tested and found deficient. Portions of the method were not written in a clear, unambiguous manner nor in sufficient detail. Therefore, it was not clear initially whether the FDA analyst's inability to use the method satisfactorily was due to inherent deficiencies in the method or inadequate description of the method. Subsequently, both types of deficiencies were found.

Food and Drug Administration chemists continued to work with Norwich to overcome the analytical difficulties until March 1972, at which time Norwich indicated that it could submit revised methods and supporting data for furazolidone. Norwich had concentrated on the tissue method for furazolidone in the belief that if the method could be worked out for that nitrofuran, adoption of the method for residues of other nitrofurans would not present any formidable problem. The revised methods were submitted April 4, 1972. Minor changes were mutually agreed upon based on an FDA review.

On May 16, 1972, steps were undertaken to proceed with laboratory validation of the methods for furazolidone tissue residues. Three government laboratories participated in the method validation. The trial involved studies of the recovery of furazolidone from homogenized samples of muscle and liver tissues from turkeys and swine to which furazolidone had been added by the analyst to achieve a drug concentration of 2 ppb (spiked tissue); and the analysis of muscle and liver tissues from swine and turkeys that had been medicated with furazolidone to produce naturally incurred residues (dosed tissues). Norwich was requested to supply dosed tissues containing residues at or near 2 ppb. According to Norwich, the dosed tissues that it supplied for the validation trial gave readings of 0.8 ppb in swine and turkey muscle tissue and 0.4 ppb in liver of both turkeys and swine based on the results uncorrected for recovery by the method of analysis undergoing trial.

The stipulated conditions of the method to be validated were that an analytical response that was at least 25% of that produced by comparison with a 2 ppb analytical standard of furazolidone would be accepted as representing a residue of furazolidone of 2 ppb or greater. This was to compensate for the inability of the method to provide for near total recovery of the residue. At that time, FDA was giving consideration to the acceptance of methods for carcinogenic drugs which were not quantitative if the reproducibility of the method was adequate and compensation could be made for the low recovery. (Twenty-five percent recovery would not be acceptable under current standards for regulatory methods of analysis.) Based on the results of the trial, FDA rejected the residue method for furazolidone.

a. Spiked tissue: The variability of recoveries from spiked tissues was too great to assure with reasonable confidence that the procedure would provide a practicable method for regulatory enforcement. The recoveries in the laboratories over all tissues ranged from 5% to 75% and did not therefore provide assurance that at least 25% recovery was consistently obtained in all tissues.

A statistical analysis was performed on the data from the spiked tissue samples to determine what percent of samples in a total population containing 2 ppb of residue may not be detected by the assay. "Detected" was defined as giving a reading of 25% or more of the reading obtained from a 2 ppb analytical standard of furazolidone as described above. In one of the government laboratories (U.S. Department of Agriculture), the percent of projected failures was computed to be (a) 43% based on dichotomous treatment of the sample data at the 95% confidence level; (b) 28% with an assumed normal distribution for the sample data and using the mean and standard deviation estimated from the sample data, or (c) 46% with an assumed normal distribution of the sample data and using a lower 95% confidence limit on the sample mean and upper 95% confidence limit on the sample standard deviation.

b. Dosed tissue: None of the three validating laboratories could verify the sponsor's findings on the dosed tissues from medicated swine or turkeys. For example, 17 analyses were conducted on turkey and swine muscle tissue stated by the sponsor to contain 2 ppb furazolidone (actual analytical reading of 0.8 ppb). Of these 17 analyses, only 2 positive findings (not corrected for recovery), i.e., 0.6 ppb and 5.1 ppb, were reported. Positive findings on these two samples could not be obtained in subsequent analyses. One laboratory obtained zero readings in three out of nine runs of the dosed muscle tissue sample where the 5.1 ppb result was found by another laboratory.

Norwich was informed by FDA in conferences of the results of the validation trial, and FDA rejected the tissue residue method for furazolidone. Norwich was informed that not only was its method deficient in terms of accuracy and precision, but that a confirmatory method was required to provide adequate identification of the residue measured by the thin layer chromatographic method. This confirmatory method requirement was considered necessary based on general experience of FDA with the occurrence of false positive results when detection and measurement techniques are used which are not inherently specific, e.g., the chromatographic techniques. The probability of occurrence of false positive results due to unpredictable interferences increases, particularly when residue measurement is necessary at extremely low levels. Furthermore, difficulties encountered in testing the assay method and replication of results indicated a need to assure specificity of the residue method.

While the validation of the 1972 method was in progress, Norwich was exploring the use of new instrumentation (programmed multiple development thin layer chromatography (PMD–TLC)) and the mechanization of spotting thin layer chromatography plates. It was believed that these new techniques, both the new instrumentation and the mechanization of spotting thin layer chromatography plates, would result in improved recovery data. Based on the new developments, Norwich requested an extension of time to complete development of the PMD–TLC method. New data using this method on chicken, turkey, swine and calves tis-

sues were submitted by sponsors in September 1974, and reviewed by FDA.

The recovery data reported by Norwich for the 2 ppb level of measurement using the PMD–TLC method were variable and not substantially improved from that previously submitted, in 1972, which had been found unacceptable in the validation trial. Therefore, the 1974 data on furazolidone were rejected as insufficient to support a new method validation trial. The data Norwich had submitted in 1972 to support conducting the validation trial only included data on swine and chicken tissues. These data showed that recoveries in swine tissues ranged from 24% to 60% and in chicken tissues from 34% to 58%. The data submitted in 1974 using the PMD–TLC method included recovery data for swine, calves, chickens and turkeys. Although these data indicate a generally higher recovery than the 1972 data, there was no meaningful improvement because of the greater variability. Norwich did not submit sample calculations. However, the FDA interpretation of the firm's data indicates, for example, the following range of recoveries for the analysis of chicken liver spiked at 2 ppb with furazolidone on 4 different days: 118%–121%, 71%–99%, 70%–146% and 48%–71%. The lowest recovery reported was with swine fat tissue where the analyses were only done on 1 day and according to the FDA interpretation of the data, ranged from 28%–90% recovery.

Norwich claims that recoveries in nonfatty tissues ranged from 36.2% to 91.5% with a mean of 62% and a relative standard deviation of 18.8%. It claimed the fatty tissues ranged from 32%–76.2% with a mean of 53.2% and a relative standard deviation of 24%. However, the basis for these conclusions is not evident from the individual data submitted. In fact, the individual data appear to contradict the conclusion because in many cases the upper range of recovery exceeded 100%, according to FDA interpretation of the data submitted. Regardless of whose interpretation is correct, the degree of accuracy and precision is not acceptable in either case.

Moreover, Norwich rejected results for about 10% of the non-fatty samples analyzed and 12% of the fatty samples analyzed because of inconsistent results which it claims were attributable to mechanical loss, contamination, high control tissue values, incorrect sample fortification, TLC spots not corresponding to the appropriate R_f values or the presence of an interfering fluorescent band. Although it may be reasonable to exclude certain outlying data from the data reduction analysis, FDA considers the rate of exclusion excessive and, in some cases, invalid and prejudicial. For example, difficulties with high control samples and interferences are indicative of potential inherent flaws in the method.

The Director concludes that practicable methods of analysis for tissue residues of furazolidone are not available. The methods provided for the determination of furazolidone are not reliable in that they are not quantitatively reliable at the levels of interest, i.e., they are not sufficiently accurate or precise, and they are not adequately specific to assure qualitative reliability. Therefore, on this basis alone, they would not be practicable for monitoring and enforcement.

Furthermore, the Director concludes that adequate information has not been provided to assure that measurement of furazolidone, as a marker, is adequate to control all potentially carcinogenic residues that may result from the use of the drug and that the methods are not adequately sensitive.

(6) *Practical Conditions of Use Cannot be Prescribed on the Label of the Product to Assure "No Residue".* Norwich has provided data which demonstrate that furazolidone is present in the edible tissues of treated animals and that a withdrawal period is required as a condition of use to assure "no residue".

A withdrawal period is the time following drug treatment when a treated animal cannot be slaughtered for food. A withdrawal period must be sufficiently long to assure adequate depletion of the residues, but it cannot be so long that it is impractical and would not likely be followed in practice. Section 512(d)(1)(H) of the act requires that for a carcinogenic drug, no residues are permitted in edible products "under the conditions of use * * * reasonably certain to be followed in practice".

It is required by FDA that residue depletion studies be conducted under simulated field use conditions to develop data for establishing the withdrawal period. An adequate analytical method for residues, preferably the same method that will be approved for regulatory use, must be used to measure the residues.

Two types of studies were used by the drug sponsors to provide information on parent drug residues of furazolidone and their depletion with time. These studies were submitted to FDA to support the adequacy of the withdrawal period.

One type of study used furazolidone which was radiolabeled with carbon-14 in the ring. A reverse isotope dilution technique was used to measure that portion of the total radioactive residue which was the parent drug. In these studies, data were not presented on the total amount of radioactive residue.

In the other type of study a chemical assay was used to measure the parent drug residue. In most of these studies, the chemical method used to measure furazolidone was very similar to the method that had been subjected to validation trials in 1972 and shown to be unreliable. All of the studies were conducted prior to initiation of the method validation studies.

Radiolabeled studies of furazolidone were conducted only with chickens and were not conducted for turkeys or swine. None of the studies measured total drug residues which the Director has concluded are necessary. A discussion of each of the studies follows.

CHICKEN STUDIES

Three studies were submitted on residue depletion of the parent drug furazolidone from medicated chickens.

1. "Furazolidone (NF–180) Residues in Chickens and Eggs," March 18 and July 7, 1969. Final Report, Problem No. 461.03–A,B, NADA 9–393, Vol. 4, submitted by cover letter to FDA January 8, 1971.

Layer rations were mixed with ^{14}C ring-labeled furazolidone at levels of 25,100 and 200 grams per ton and fed to three groups of hens for 14 days. The maximum use approved for the drug is 200 grams per ton for 21 days. Birds were sacrificed on the 14th day of medication (zero day withdrawal) and at 3 and 5 days following withdrawal from medication. Samples of liver, kidney, muscle and skin with fat from six birds sacrificed at 0 and 3 days withdrawal time were analyzed.

The sponsor claimed its radiotracer method was capable of measuring furazolidone as low as 2 ppb via a reverse isotope dilution technique. Using this method, the sponsor says that the concentration of furazolidone was 13 ppb in skin with fat samples at zero withdrawal from birds fed 200 grams per ton. Furazolidone was not detectable in all other tissues at zero withdrawal or detectable in any tissues after 3 days withdrawal.

2. "Direct Extraction Procedure For Furazolidone (NF–180) in Chicken, Swine and Turkey Tissues at 2 ppb," September 2, 1971, Project 475.17, Interim Report No. 5, NADA 9–393, Vol. 5.

The report contains the residue results obtained by chemical analysis for furzolidone in edible tissues of chickens, turkeys and swine. Results from the turkey and swine studies are discussed separately under the appropriate subheadings.

In the residue depletion study for chickens, furazolidone was fed at 200 grams per ton for 14 days. The report presents the tissue residue values of furazolidone in liver, muscle, kidney, and skin with fat from birds sacrificed at withdrawal times of 1, 4, and 5 days. At zero withdrawal, furazolidone was reported in skin with fat samples as 4.7 ppb and 7.1 ppb. Furazolidone was reported in one muscle sample as 2 ppb at zero withdrawal; however, the sponsor admitted uncertainty about this finding. Skin with fat samples at 4 days withdrawal are reported as 2 ppb in one sample and a questionable result in another sample. The report indicated that all other samples at the various withdrawal times did not contain detectable levels of furazolidone. The drug sponsor claimed the chemical assay was sensitive to 2 ppb.

3. "Furazolidone Depletion Studies in Chickens," Memorandum from Dr. J. P. Heotis to Mr. G. Gever, May 3, 1971, NADA 9–393, Vol. 5. From cover letter of May 4, 1971 to Lakata (FDA).

The memorandum includes the results of a tissue residue study in chickens administered 200 grams per ton of furazolidone in their diets for an unspecified time period. A chemical assay for furazolidone was used to measure the residues, and the sponsor claimed the method was sensitive to 2 ppb. Furazolidone was reported in muscle as 6.5 ppb and in liver as 2 ppb at zero withdrawal.

No data were given for skin with fat at zero withdrawal. Liver, kidney, muscle and skin with fat samples did not contain detectable furazolidone on days 3, 4, and 5 post-withdrawal.

Data are not included in this notice on residues in eggs because the firm admits that the use of furazolidone in laying hens results in significant residues in eggs. For this reason the firm has voluntarily deleted from the labeling all uses in laying hens and in replacement chickens over 14 weeks of age.

The Director concludes on the basis of the data available on tissue residues of furazolidone in chickens that:

(a) The studies are not adequate to determine total drug-related residues that may occur, because the assays used detected only the parent drug.

(b) The chemical method used for furazolidone is not reliable at the lower concentrations in tissue.

(c) Furazolidone was present in edible tissues following medication when no withdrawal period was observed.

(d) A withdrawal period after which no residues are found in edible tissues cannot be established from the information available.

TURKEY STUDIES

Three studies were submitted on residue depletion of parent drug furazolidone from medicated turkeys.

1. "Direct Extraction Procedure for Furazolidone (NF–180) in Chickens, Swine and Turkey Tissues at 2 ppb," September 2, 1971, Project 475.17, Interim Report No. 5, NADA 9–393, Vol. 5.

The report presents the tissue residue values for turkeys fed the maximum approved dose of furazolidone, i.e., 200 grams per ton for 21 days. Samples of liver, kidney, muscle, and skin with fat from two birds sacrificed at each of the withdrawal times of 0, 4 and 5 days were analyzed by a chemical method for furazolidone which the sponsor claimed was sensitive to 2 ppb. At zero withdrawal, residues in muscle are reported as 6.4 ppb and 7.7 ppb, and in skin with fat as 6.5 ppb and 8.3 ppb. Residues in skin with fat were the most persistent. At 5 days withdrawal, residues in skin with fat were reported as 2 ppb in one sample and a questionable result in another sample.

2. "Furazolidone Depletion Studies in the Turkey," November 9, 1971, Project 475.17, Interim Report No. 6, NADA 9–393, Vol. 5.

These studies provide data on tissue residues in turkeys using a chemical method for furazolidone which the sponsor claimed was sensitive to 2 ppb. In the first study birds were fed furazolidone at 100 grams per ton in their feed for 28 days. Liver, kidney, and muscle samples from birds sacrificed at 5 and 6 days withdrawal were analyzed and no furazolidone was detectable in any of the samples. Skin with fat samples from birds sacrificed at 5, 6, 7, 8 and 9 days after withdrawal were analyzed. At 5 days withdrawal, five samples were reported to contain 3 ppb of furazolidone and one sample contained 2 ppb. At 9 days withdrawal, one sample contained 2 ppb, two samples were questionable, and three samples were reported as not containing any detectable levels of furazolidone.

In a second study, birds were fed furazolidone at 200 grams per ton in their feed for 21 days. Liver, kidney and muscle tissues did not have detectable levels of furazolidone at 5 and 6 days withdrawal. All six samples of skin with fat examined at 5 days withdrawal contained concentrations of furazolidone reported at 2 ppb. At 9 days withdrawal, the longest period examined, one skin with fat sample was reported to contain furazolidone at 2 ppb and 11 samples were questionable.

3. "Furazolidone Depletion Studies in Turkey Skin with Fat," February 10, 1972, Project 475.17, Interim Report No. 7, NADA 9–393, Vol. 16.

The two studies in this report contain data on the depletion of furazolidone from skin with fat tissue only of turkeys fed furazolidone at 200 grams per ton of feed for 21 days, and at 100 grams per ton of feed for 28 days. A chemical method of analysis for furazolidone which the sponsor claimed was sensitive to 2 ppb was used. At the maximum approved feeding level of 200 grams per ton, furazolidone in skin with fat samples was reported to deplete from 7 to 8 ppb at zero withdrawal to 2 ppb at 9 days withdrawal. Furazolidone continued to be found at 2 ppb through 13 days after withdrawal of the birds from medication. When furazolidone was fed at 100 grams per ton of feed for 28 days, measurements of furazolidone in skin with fat samples were made from day 9 through day 15 following cessation of drug treatment. Furazolidone was reported at 2 ppb on day 9 and was not detected from day 12 through 15.

The Director concludes on the basis of these data on tissue residues of furazolidone in turkeys that:

(a) The studies are not adequate to determine the total drug-related residues that may occur, because the chemical assay used detected only residues of the parent drug.

(b) The chemical method used for furazolidone is not reliable at the lower concentrations in tissue, and therefore the residue data on the depletion of furazolidone are not acceptable.

(c) Furazolidone was found in edible tissues following medication and continued to persist in skin and fat even after withdrawal from medication for 5 days.

(d) A withdrawal period after which no residues are found in edible tissues cannot be established from the information available.

SWINE STUDIES

Three studies were submitted on residue depletion of parent drug furazolidone from medicated swine.

1. "Summary of Pertinent Data, Furazolidone, Residue Data," January 8, 1971, Tab C, NADA 9–393, Vol. 4.

Residue studies using a chemical method of analysis for furazolidone which the sponsor claimed was sensitive to 0.1 ppm are reported. In one study, swine were fed 300 grams per ton of furazolidone in their diets for 7 months. (The maximum approved use of furazolidone is 300 grams per ton for 14 days.) All tissues, i.e., muscle, liver, kidney, and fat, were reported as containing no detectable levels of furazolidone at zero withdrawal. In another study swine were fed the drug in feed at 200 grams per ton for 6 months along with antibiotics. Again, no detectable levels of furazolidone were found at zero days withdrawal.

2. "Tissue Residue Studies for Furazolidone in Swine," Memorandum from Dr. J. L. Mertz to Dr. J. P. Heotis, October 4, 1971, NADA 9–393, Vol. 5. From cover letter of October 5, 1971.

The memorandum describes studies in which swine were fed furazolidone at a level of 300 grams per ton in feed for 21 days. Samples of kidney, liver, muscle, and fat from animals sacrificed at 0, 4, 5 and 6 days after withdrawal from medication were examined. A chemical method of analysis for furazolidone was used which the sponsor claimed was sensitive to 2 ppb. The only kidney sample examined at zero withdrawal was reported to contain furazolidone at 2 ppb. Two fat samples contained 2 ppb and one fat sample contained 5 ppb at zero withdrawal time. All other tisseus at zero withdrawal did not contain detectable levels of furazolidone. At all other withdrawal times, all tissues with the exception of fat did not contain detectable levels of furazolidone. One fat sample at 4 days withdrawal was reported to contain furazolidone at 2 ppb. Furazolidone was not found in any fat samples taken from animals at 5 and 6 days withdrawal from medication.

3. "Direct Extraction Procedure for Furazolidone (NF–180) in Chicken, Swine and Turkey Tissue at 2 ppb," September 2, 1971, Project 475.17, Interim Report No. 5, NADA 9–393, Vol. 5.

In this study, swine were fed furazolidone at a level of 150 grams per ton in feed for 35 days. Using a chemical method of analysis for furazolidone which the sponsor claimed was sensitive to 2 ppb, only fat was reported to contain furazolidone at zero withdrawal. All tissues, i.e., liver, muscle, kidney and fat, did not contain detectable levels of furazolidone at 4 as well as 5 days withdrawal.

The Director concludes on the basis of the data available on tissue residues of furazolidone in swine that:

(a) The studies are not adequate to determine the total drug-related residues that may occur, because the chemical assay used detected only residues of the parent drug.

(b) The chemical method used for furazolidone is not reliable at the lower concentrations in tissue.

(c) Furazolidone was found in edible tissues following medication when no withdrawal period was observed.

(d) A withdrawal period when no furazolidone is found in the tissues cannot be established from the information available.

The Director concludes that information has been provided showing that with-

drawal periods are required for the safe use of furazolidone. He concludes that essential information has not been provided to establish withdrawal periods which are adequate to assure no residues and are practical within the meaning of the act. He further concludes from the available data that the currently labeled withdrawal periods for turkeys are not adequate in that residues of furazolidone have been found in skin with adhering fat for days after the currently labeled withdrawal period. He also concludes on the basis of available data that it is unlikely that the currently labeled withdrawal period for swine and chickens would be shown adequate if proper studies were available which took into account total drug residues.

Therefore, the Director of the Bureau of Veterinary Medicine is hereby withdrawing the notice of opportunity for hearing issued August 4, 1971, proposing to withdraw approval of NADA's for the use of furazolidone in food-producing animals. Under section 512(e)(1) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b,(e)(1)), the Director proposes to withdraw approval of certain existing NADA's providing for the use of furazolidone in food-producing animals on the grounds that such use violates section 512(d)(1)(H) of the act.

This notice applies to all outstanding NADA's for the use of furazolidone in food-producing animals. All such applications now known to FDA are listed below by NADA number and by each applicant's name and last known address in FDA records. Copies of this notice are being forwarded by registered or certified mail to each of the known applicants. Notice to other applicants or holders of such NADA's whose identity is not known to FDA is given by this publication.

9-073, 11-405, 11-698, 12-061, Hess and Clark, Division of Rhodia, Inc., 7th and Orange Street, Ashland, OH 44805.

9-393, 13-805, Norwich Pharmacal Co., Division of Morton-Norwich Products, Inc., 13-27 Eaton Avenue, Norwich, NY 13815.

11-016, 11-810, 32-319, Eaton Laboratories, Division of Morton-Norwich Products, Inc., P.O. Box 191, Norwich, NY 13815.

Any holder of an approved application that elects to avail itself of the opportunity for hearing pursuant to section 512(e)(1) of the act and § 514.200 of Title 21 of the Code of Federal Regulations must file with the Hearing Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville MD 20852, a written appearance requesting such hearing by June 14, 1976, giving the reasons why approval of the application should not be withdrawn, and providing a well-organized and full-factual analysis of the scientific and other investigational data such holder is prepared to prove in support of its opposition to the Director's proposal. A request for hearing may not rest upon mere allegations or denials but must set forth specific facts showing that there is one or more genuine and substantial issues of fact requiring a hearing. Responses to this notice may be seen in the office of the Hearing Clerk during regular business hours, Monday through Friday.

If a hearing is requested and is justified by any holder's response to this notice of opportunity for hearing, the issues will be defined, an Administrative Law Judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

Any hearing on the withdrawal of approval of the foregoing NADA's will be open to the public. If, however, the Director finds that portions of the applications that serve as a basis for such a hearing contain information concerning a method or process that is entitled to protection as a trade secret, the part of the hearing involving such portions will not be public, unless the respondent so specifies.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (section 512, 82 Stat. 343-361 (21 U.S.C. 360b)) and under authority delegated to the Commissioner (21 CFR 2.120) and subdelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 2.121(m), published in the FEDERAL REGISTER of January 19, 1976 (41 FR 2636)).

A discussion of the economic and environmental impact of this action is set forth in the advanced notice of proposed rule making on furazolidone published elsewhere in this issue of the FEDERAL REGISTER.

It is hereby certified that the economic and inflation effects of this proposal have been carefully evaluated in accordance with Executive Order 11821.

Dated: May 10, 1976.

C. D. VAN HOUWELING,
Director,
Bureau of Veterinary Medicine.

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